DEVELOPMENT AND CLINICAL TRANSLATION OF MICRONEEDLES FOR INSULIN DELIVERY AND SELF-VACCINATION

A Dissertation
Presented to
The Academic Faculty

by

James Jefferis Norman

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy in the
School of Chemical & Biomolecular Engineering

Georgia Institute of Technology
December 2012

DEVELOPMENT AND CLINICAL TRANSLATION OF MICRONEEDLES FOR INSULIN DELIVERY AND SELF-VACCINATION

Approved by:

Dr. Mark Prausnitz, Advisor
School of Chemical & Biomolecular Engineering
Georgia Institute of Technology

Dr. Mark Allen
School of Electrical & Computer Engineering
Georgia Institute of Technology

Dr. Eric Felner
School of Medicine
Division of Pediatric Endocrinology
Emory University

Dr. Paula Frew
Rollins School of Public Health
School of Medicine
Emory University

Dr. Martin Meltzer
Office of Infectious Diseases
National Center for Emerging and Zoonotic Infectious Diseases
Centers for Disease Control and Prevention

Date Approved: November 2, 2012

To my family, for encouraging me to finish

ACKNOWLEDGEMENTS

I could not have completed this dissertation without the support of the people listed here. First, I would like to thank my thesis advisor, Dr. Mark Prausnitz for accepting me into his world-class research group and advising me for six years. I was quite naïve when I entered the lab, but he took a growth mindset and transformed my naïveté into a space for professional growth.

As for my other committee members, they each contributed to my education and to this dissertation. Dr. Allen introduced me to graduate research and taught me how to ask insightful questions, Dr. Felner introduced me to clinical research, Dr. Frew introduced me to the psychological research and participant recruitment, and Dr. Meltzer introduced me to life at the CDC and quantitative health economics.

Thank you to the graduate students, post-docs, and research scientists I worked with in the Prausnitz and Allen groups: Dr. Vladimir Zarnitsyn, Dr. Samir Patel, Dr. Saffar Mansoor, Dr. Jyoti Gupta, Chris Edens, Matt Mistilis, Seonhee Park, Dr. Leonard Chu, Josh Hutchison, Dr. Sean Sullivan, Dr. Jung-Hwan Park, Dr. Avishek Aiyar, Dr. Jason Jiang, Dr. Tanicia Daley, Winston Pewin, Sebastien Henry, Dr. Devin McAllister, Dr. Myke Scoggins, Dr. Yeu-Chun Kim, Dr. Hyo-Jick Choi, Dr. Young Bin Choy, Dr. Xin Dong Guo, Dr. Seong-O Choi, Dr. Jeong-Woo Lee, Dr. Robyn Schlicher, Dr. Hang Jun Park, Dr. Ying Liu, Dr. Samantha Andrews, Dr. Prerona Chakravarty, Dr. Priya Kalluri, Aritra Sengupta, Yoo-Chun Kim, Jaya Arya, and Pradnya Samant. These 30+ co-workers are good, helpful friends.

Several Georgia Tech staff members helped with this research as well. Donna Bondy deserves the most credit. She handles a difficult job, yet still has time for lighthearted converstations. Allen Echols, Steve Woodard, James Goddard, and Alyceson Andrews managed IBB well. Richard Shafer helped with laser cutting and various other fabrication issues. Brad Parker, Jeff Andrews, and Dennis Brown machined devices for this research. The PRL staff helped with *in vivo* studies. Off campus, I worked extensively with two suppliers – Andrew Wilt at Permegear and Alan Iranian at Cartika – who deserve thanks for meeting deadlines on unusual requests.

The following undergraduate researchers put in some significant hours for this research: Brian Bondy, Nhien Tong, HyeSun Jun, and Betul Benzer. I wish them luck in the future careers, though I hardly need to.

Thank you to Dr. Milton Brown, Nick Raviele, and Alleia Stokes for their roles in the insulin study at Emory Children's Center. Thank you to Marc Padilla, Marcus Bolton, and Brooke Hixson at the Hope Clinic and Dr. Maxine McClain for the help with participant recruiting and study conduct. Thank you to Darin Zehrung, Courtney Jarrahian, Emily Griswold, Sarah McGray, Erica Jacoby, and others at PATH for their advice and work on the self-administration studies. Thank you to Dr. Bruce Weniger, formerly at the CDC, for sparking my interest in global health. Thank you to Dr. Alan Hinman at Emory University for initially advising the research on self-vaccination.

Thank you to all of the participants in the research studies. Thank you to the IRB and IACUC for overseeing the responsible conduct of this research. Thank you to the funding agencies: the National Institutes of Health, the Thrasher Research Fund, the Department of Education, and the Georgia Institute of Technology.

Thank you to the people outside of work who helped with personal development and work-life balance. Michael Spinner brought out my dedication and ability to finish difficult tasks. Dr. Nelson Binggeli helped me realize my full potential in research. Thank you to my family for their love, encouragement, and support. Lastly, thank you to my wonderful wife, Sharon for providing love, friendship, and support.

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS iv

LIST OF TABLES xii

LIST OF FIGURES xiii

LIST OF SYMBOLS xv

LIST OF ABBREVIATIONS xvi

SUMMARY xviii

CHAPTERS

1 INTRODUCTION 1

1.1 Motivation 1

1.2 Microneedles for Insulin Delivery and Self-Vaccination 2

1.3 Specific Aims 3

1.3.1 Fabricate a side-opened, sharp-tipped, hollow metal microneedle using micromolding and characterize the microneedles *in vitro* and *in vivo* 3

1.3.2 Compare insulin delivery using microneedles and subcutaneous catheters in children and adolescents in a phase I randomized trial 3

1.3.3 Conduct a preliminary human study to evaluate the usability, acceptability, and cost-effectiveness of microneedle patches for self-vaccination against influenza 3

1.4 Outline of Remaining Chapters 4

2 BACKGROUND 5

2.1 Drug Delivery of Biopharmaceuticals 5

2.1.1 Definition of Biopharmaceuticals 5

2.1.2 General Unavailability of Oral and Transdermal Delivery 5

2.1.3 Hypodermic Needle Injections 6

2.2 The Skin as a Drug Delivery Target for Biopharmaceuticals 7

2.2.1 Methods for Transcutaneous Drug Delivery 7

2.2.2 Rapid Systemic Uptake and Improved Access to Immune System 8

2.3 Microneedles 9

2.3.1 General Design Criteria for Microneedles 11

2.3.1.1 Solid Microneedles 12

2.3.1.2 Coated Microneedles 12

2.3.1.3 Dissolving Microneedles 13

2.3.1.4 Hollow Microneedles 13

2.4 Insulin Delivery 14

2.4.1 Diabetes 14

2.4.2 Insulin Therapy for Type 1 Diabetic Patients 15

2.4.3 Approved Insulin Administration Methods 16

2.5 Vaccine Delivery 17

2.5.1 Introduction to Vaccines 18

2.5.2 Approved Administration Methods 19

2.5.3 Vaccination Settings 20

2.6 Behavioral Analysis with the Theory of Reasoned Action 20

2.6.1 Method for Generating and Analyzing Data for a Theory of Reasoned Action Model 22

2.6.2 Example Behavioral Studies Concerning Vaccine Acceptance 23

2.7 Economic Analysis of Vaccination 23

2.7.1 Economic Analysis of Vaccination Devices 26

3 MOLDING PROCESS FOR HOLLOW METAL MICRONEEDLES 27

3.1 Introduction 27

3.2 Device Fabrication 28

3.2.1 Master Structure 29

3.2.2 Replicating the Master Structure 30

3.2.3 Seed Layer and Electrodeposition 31

3.2.4 Dissolving the Sacrificial Replicate Structure 33

3.2.5 Device Assembly 33

3.2.6 Final Device Imaging 34

3.3 Force Displacement Measurement 35

3.3.1 Experimental Results 35

3.4 Insertion and Injection into Pig Skin *In Vitro* 36

3.4.1 Insertion into Pig Skin 36

3.4.2 Injection into Pig Skin 37

3.5 Insertion and Injection into Hairless Guinea Pig Skin *In Vivo* 37

3.5.1 Experimental Results 38

3.6 Discussion 39

4 INSULIN DELIVERY USING MICRONEEDLES IN TYPE 1 DIABETIC CHILDREN AND ADOLESCENTS 43

4.1 Introduction 43

4.2 Research Design and Methods 45

4.3 Results and Discussion 49

5 USABILITY, ACCEPTABILITY, AND COST-EFFECTIVENESS OF MICRONEEDLE PATCHES FOR SELF-VACCINATION AGAINST INFLUENZA 58

5.1 Introduction 58

5.2 Methods 61

5.2.1 Microneedle Patch Fabrication 61

5.2.2 Study Approval and Participant Recruiting 61

5.2.3 Experimental Procedures 63

5.2.4 Skin Staining to Measure Usability 63

5.2.5 Questionnaires to Measure Acceptability 64

5.2.6 Quantitative Economic Analysis to Assess Cost-Effectiveness 64

5.3 Results 67

5.3.1 Usability 67

5.3.2 Acceptability 69

5.3.3 Cost-Effectiveness 72

5.3.4 Pain 75

5.3.5 High Effectiveness Patch: Acceptability and Cost-Effectiveness 76

5.3.6 Factors Affecting Microneedle Patch Uptake 78

5.4 Discussion 80

6 CONCLUSIONS 83

6.1 Fabrication of Hollow Metal Microneedles 83

6.2 Insulin Delivery Using Microneedles To Type 1 Diabetic Children and Adolescents 83

6.3 Usability, Acceptability, and Cost-Effectiveness of Microneedle Patches for Self-Vaccination against Influenza 84

7 RECOMMENDATIONS 85

7.1 Recommendations for Hollow Microneedle Design 85

6.2 Recommendations for Insulin Delivery to Human Subjects 85

6.3 Recommendations for Self-Vaccination 86

6.1 General Recommendations for Microneedles 86

APPENDIX A: DETAILED METHODS 89

APPENDIX B: FORMS USED IN HUMAN STUDIES 121

REFERENCES 144

LIST OF TABLES

Page

Table 3.1: Effect of Starting Condition on Electrodeposition Process Yield 32

Table 4.1: Participant Demographics (insulin study) 50

Table 5.1: Participant Demographics (self-vaccination study) 62

Table 5.2: Inputs of the Economic Model – Illness Parameters and Costs 65

Table 5.3: Inputs of the Economic Model – Disease Rates 66

Table 5.4: Results of Factor Analysis 79

LIST OF FIGURES

Page

Figure 2.1: Methods of drug delivery to the skin using microneedles 10

Figure 2.2: Example image for each type of microneedle 10

Figure 2.3: Time-action profiles of insulin lispro (Humalog) and regular human insulin (Humulin R), both administered subcutaneously 16

Figure 2.4: Schematic of the theory of reasoned action 21

Figure 3.1: Fabrication Sequence 29

Figure 3.2: Optical microscopy images of the microneedle device 34

Figure 3.3: Representative force-displacement curve for a microneedle pressed against a rigid surface 35

Figure 3.4: Microneedle injection into pig skin *in vitro* 36

Figure 3.5: Microneedle injection into hairless guinea pig skin *in vivo* 39

Figure 4.1: Microneedle used for intradermal insulin delivery 47

Figure 4.2: Pain of microneedle and subcutaneous insulin administration 51

Figure 4.3: Pharmacokinetics and pharmacodynamics of insulin delivery 52

Figure 4.4: Pharmacokinetic analysis of intradermal insulin delivery using microneedles 53

Figure 5.1: Microneedle patch and examples of skin stains 67

Figure 5.2: Usability of microneedles versus administration attempt 68

Figure 5.3: Acceptability of microneedles and self-vaccination 70

Figure 5.4: Acceptability of microneedles and self-vaccination, equal effectiveness 71

Figure 5.5: One-way sensitivity analysis for the change in total payer costs after introducing self-administered microneedles 73

Figure 5.5: Two-way sensitivity analysis plotting additional cost for payer to improve coverage by 19% 74

Figure 5.7: Paired comparison of pain scores 75

Figure 5.8: Acceptability of high-effectiveness microneedles and self-vaccination 76

Figure 5.9: Comparison of cost-effectiveness histograms for different scenarios 76

LIST OF SYMBOLS

a	minimum (triangular distribution)
b	maximum (triangular distribution)
c	mode (triangular distribution)
k_a	absorption coefficient
k_e	elimination coefficient
p	probability of a statistical result
CO_2	carbon dioxide
U	standard unit of insulin
Δ	delta / change in
μ	mean (distribution), micro (as a prefix)
σ	standard deviation

LIST OF ABBREVIATIONS

ACIP	Advisor Committee on Immunization Practices
AUC	area under the insulin curve
BCG	Bacillus Calmette- Guérin vaccine
BMI	body mass index
CBA	cost-benefit analysis
CDC	Centers for Disease Control and Prevention
CEA	cost-effectiveness analysis
CI	95% confidence interval
CSII	continuous subcutaneous insulin infusion
DALY	disability adjusted life year
FDA	United States Food and Drug Administration
GRAS	generally regarded as safe
HbA1C	glycated hemoglobin
HCW	healthcare worker
HIV	human immunodeficiency virus
IA	investigator administration
IACUC	institutional animal care and use committee
IM	intramuscular
IRB	institutional review board
LAIV	live attenuated influenza vaccine
LED	light-emitting diode
MN	microneedle
MNs	microneedles

NPH	neutral protamine Hagedorn
OR	odds ratio
PBS	phosphate buffered saline
PDMS	poly(dimethylsioxane)
PLA	poly(lactic acid)
QALY	quality adjusted life year
SA	self-administration
SC	subcutaneous
SIC	subcutaneous infusion catheter
US	United States
VAS	visual analog scale
WHO	World Health Organization

SUMMARY

Type 1 diabetes and influenza cause significant illness and unnecessary medical costs despite the existence of insulin for maintenance of diabetes and a vaccine for prevention of influenza. This dissertation describes three studies on the development and clinical translation of microneedles to improve the administration of these biopharmaceuticals.

The first study reports on a sharp-tipped hollow metal microneedle designed to reduce manufacturing costs, improve insertion into skin, and improve fluid flow compared to other hollow microneedles used for drug delivery. The results showed sharp-tipped metal microneedles could be fabricated using an inexpensive electroplating and sacrificial micromolding process. Single-microneedle devices made by this method achieved high flow rates and delivered model drugs into tissue.

The second study reports on insulin delivery using microneedles in children with type 1 diabetes. The results showed microneedle insertion was less painful, which is a promising result for improving injection compliance in children. Additionally, microneedle delivery showed rapid onset of insulin action compared to subcutaneous catheter delivery, which may enable automatic closed-loop insulin therapy. This was the first study of drug delivery to children using microneedles.

The last study reports on microneedle patches for self-vaccination against influenza. Human subjects were recruited from greater Atlanta, were asked to self-administer placebo microneedle patches, and were then given a dynamic questionnaire to determine their views and preferences regarding influenza vaccination using

microneedles compared to conventional intramuscular injection. The results showed that microneedles were usable by the participants, the introduction of microneedles may improve vaccination coverage by approximately 20%, and self-administration of vaccines may significantly reduce vaccination costs for a healthcare payer. This was the first study to assess the ability of human subjects to self- administer a microneedle patch and the first study to determine the potential impact of self-vaccination against influenza using a microneedle patch on vaccination coverage and vaccination cost.

Overall, the fabrication advances and positive findings from human subjects research support additional translation of microneedles for insulin delivery and self-vaccination toward clinical use.

CHAPTER 1

INTRODUCTION

1.1 Motivation

Insulin and influenza vaccines have been administered to millions of patients over several decades, but for both these important medicines, significant delivery challenges remain.

For insulin delivery to type 1 diabetic patients, the challenge is twofold. First, many patients fear injections and this fear of injection correlates with worse glycemic control and health outcomes [1-3]. Second, insulin uptake after subcutaneous delivery is too slow to enable automated control of blood glucose [4], leaving patients with the significant responsibility for monitoring and managing their diabetes.

For influenza vaccination, the challenge is improving vaccination coverage while constraining costs. The United States has a universal influenza vaccination recommendation, but coverage levels fall well below target due to reasons such as injection phobia and inconvenience of vaccinations [5]. As a result, the United States has more than 200,000 hospitalizations and 3,000-49,000 deaths due to influenza each year [6]. Expanding influenza coverage is desirable but brings cost-effectiveness concerns, particularly for healthy adults age 18-49, the last group recommended for influenza vaccination. The cost effectiveness of influenza vaccine delivery is primarily limited by administration and patient time costs, which outweigh the cost of the vaccine itself 3.3 to 1 [7].

1.2 Microneedles for Insulin Delivery and Self-Vaccination

Microneedles, needles less than 1.5 mm in length used for skin delivery of drugs, may help solve the challenges of insulin and influenza vaccine delivery. Drugs such as insulin and influenza vaccines, normally impermeable in skin, can be precisely injected as liquids using hollow microneedles or as solids with coated or dissolving microneedles [8].

For insulin delivery, microneedles are expected to reduce injection fear because the needles are barely perceptible. Additionally, microneedle trials completed to date have shown microneedles consistently reduce needle-insertion pain compared to traditional injection methods [9]. Studies in adults have also shown that microneedle-mediated intradermal delivery accelerates insulin pharmacokinetics [10, 11], potentially enabling closed-loop control of blood glucose.

Microneedles may benefit influenza vaccination not just by reducing needle-phobia, but also by enabling self-administration of vaccines. Currently, only one self-administered vaccine exists, the oral typhoid vaccine [12]. Using microneedles to expand self-vaccination to the more commonly used influenza vaccine could improve convenience for patients, improve coverage, and significantly reduce non-vaccine-related costs.

1.3 Specific Aims

1.3.1 Fabricate a side-opened, sharp-tipped, hollow metal microneedle using micromolding and characterize the microneedles *in vitro* and *in vivo*

The purpose of this study was to integrate several desirable features of hollow microneedles into a single design with a simple manufacturing process. The resulting microneedles were characterized with destructive testing as well as fluorescent dye delivery in pig and hairless guinea pig skin.

1.3.2 Compare insulin delivery using microneedles and subcutaneous catheters in children and adolescents in a phase I randomized trial

To improve children's compliance with insulin therapy and accelerate insulin pharmacokinetics, this study tested the hypothesis that intradermal insulin delivery using microneedles causes less insertion and infusion pain and leads to faster onset (time to peak concentration) and offset (time to fall to half of peak concentration) of insulin pharmacokinetics in children with type 1 diabetes compared to conventional subcutaneous injection. In this repeated measures study, 16 pediatric type 1 diabetic patients participated and experienced microneedle and subcutaneous administration of insulin on separate days.

1.3.3 Conduct a preliminary human study to evaluate the usability, acceptability, and cost-effectiveness of microneedle patches for self-vaccination against influenza

We conducted a randomized, repeated measures trial to assess whether a self-administered microneedle patch is usable, acceptable, and more cost-effective for improving vaccination coverage compared to intramuscular injection. Seventy healthy adults experienced three self-administrations, one investigator-administration, and a

control intramuscular injection. We measured usability with skin staining and acceptability with a stated preference questionnaire. A quantitative economic model evaluated cost-effectiveness in terms of additional payer costs needed to improve vaccination coverage. Prior to this study, there were no data on self-vaccination using microneedle patches and limited information comparing self-administration to healthcare-worker-administration for other vaccination methods [13-15], but no studies assessing coverage improvements due to self-administration or cost-effectiveness for equivalent vaccines.

1.4 Outline of Remaining Chapters

Chapter 2 contains background reading on microneedles, the drug delivery problems motivating this work, and the methods used in this dissertation; chapters 3-5 present work done on the three specific aims; chapter 6 concludes the dissertation; chapter 7 provides recommendations for future work on microneedles.

CHAPTER 2

BACKGROUND

2.1 Drug Delivery of Biopharmaceuticals

2.1.1 Definition of Biopharmaceuticals

Biopharmaceutical, as a term, has several meanings. This dissertation will use the following recommended definition: a biopharmaceutical is "a pharmaceutical inherently biological in nature and manufactured using biotechnology" [16]. Biopharmaceuticals include proteins, polysaccharides, nucleic acid chains, as well as larger structures such as viruses and bacteria.

2.1.2 General Unavailability of Oral and Transdermal Delivery

Oral delivery and transdermal patch delivery are two well-accepted, low-cost means of delivering drugs. The oral route is unavailable for most biopharmaceuticals due to degradation in the gastrointestinal tract or limited systemic uptake [17]. Current exceptions are rare and include small cyclic proteins [18], vaccines targeted against orally transmitted pathogens (polio, typhoid, cholera, and rotavirus), and one oral adenovirus vaccine [19].

Delivery of drugs across intact skin using transdermal patches is also unavailable for most biopharmaceuticals because of drug size restrictions. The uppermost layer of skin, a 15μm layer called the stratum corneum, is a diffusion barrier that prevents water loss and entry of foreign molecules. Drug delivery across the stratum corneum is limited

to small, lipophilic compounds that can diffuse through the lipid bilayers of the barrier's cells [20, 21]. Drugs amenable to transdermal delivery are all less than 500 Da in molecular weight and approximately 1 nm in diameter [22]. Typical biopharmaceuticals are much larger. Insulin has a molecular weight of approximately 6000 Da, depending on the specific amino acids. Influenza vaccines range from single proteins with molecular weights of 56,000 Da [23] to whole viruses 100 nm in diameter. These large biopharmaceuticals are unable to cross an intact diffusion barrier of the skin for significant therapeutic effect.

2.1.3 Hypodermic Needle Injections

Because the oral and transdermal routes are unavailable, the majority of biopharmaceuticals are administered by injection with a hypodermic needle. As mentioned in [8], "[injections provide] a low-cost, rapid, and direct way to deliver almost any type of molecule into the body." Drugs can be injected into numerous tissues, but the most common injections for biopharmaceuticals are subcutaneous and intramuscular injections. The subcutaneous space is the fat layer below the skin, and it is a common injection site for insulin [24] and vaccines [25]. Intramuscular injections deliver fluid to muscles below the subcutaneous space. Inactivated influenza vaccines are traditionally given intramuscularly [25]. Another, less common administration route is intradermal injection, injection of fluid into the skin. Intradermal injections are used to administer certain biopharmaceuticals such as tuberculin and the rabies vaccine [26]. Intradermal injections using hypodermic needles are uncommon because they require shallow, angled injections that are difficult to perform [27].

Injections, although widely used, have limitations. These include: 1) pain and fear that reduce patient compliance [1, 28], 2) the restrictions on self-administration due to the need for patient training and safe needle disposal, and 3) the difficulty targeting drugs anywhere except deep into a tissue.

2.2 The Skin as a Drug Delivery Target for Biopharmaceuticals

Several new drug delivery techniques are in development to replace hypodermic needle injections for delivery of large biopharmaceuticals. Drug delivery to the skin, also known as transcutaneous delivery, overcomes the limitations of hypodermic needle injections: 1) patients mostly perceive skin delivery methods as less painful than injections [21, 29]; 2) skin delivery methods may enable safe self-administration of biopharmaceuticals [21, 29]; and, 3) skin delivery enables targeting to the circulatory system for rapid drug uptake and the immune system for improved immunotherapeutics.

2.2.1 Methods for Transcutaneous Drug Delivery

A review of transcutaneous drug delivery methods is available from Kim et al. [29]. Traditional techniques for drug delivery to the skin include intradermal delivery [27] (see section 2.1.3) and scarification. Scarification involves poking a liquid formulation into the skin with a short needle like a bifurcated needle [30]. Modern techniques project a drug into the skin, inject a drug into the skin, or modify skin permeability to enable patch delivery of biopharmaceuticals. Projectile methods include jet injection [31] and gene gun delivery [32]. Injection methods include tattoo gun delivery [33] and delivery with coated, dissolving, and hollow microneedles [34]. Methods for modifying skin permeability for patch application of biopharmaceuticals

include solid microneedle application [35], skin abrasion [36], ultrasound [37], electroporation [38], chemical enhancers [39], and thermal ablation [40]. This dissertation focuses primarily on microneedles, and the rationale for using microneedles over other skin delivery techniques is discussed in section 2.3.

2.2.2 Rapid Systemic Uptake and Improved Access to the Immune System

Biopharmaceuticals administered to the skin, including insulin [10, 11, 41, 42], parathyroid hormone [43], and etanercept [44], have shown rapid systemic uptake (rapid pharmacokinetics) compared to traditional injection methods such as subcutaneous delivery. The vascular and lymphatic networks in the skin are higher in density and more permeable compared to the vascular and lymphatic networks in the subcutaneous space [45, 46]. It is hypothesized that large biopharmaceuticals have enhanced pharmacokinetics due to rapid lymphatic uptake [44]. Whether the rapid uptake is beneficial for a biopharmaceutical's action in the body, its pharmacodynamics, depends on the drug.

In addition to enhanced pharmacokinetics, the skin also provides enhanced access to the immune system compared to other injection sites [44, 47, 48]. For vaccines in particular, skin immunization can lead to improved immune responses (ex: improved antibody titers, memory, or cross-protection) at equal doses [49-51] or equivalent immune responses at reduced doses [52-54]. The improved vaccine responses are thought to result from efficient targeting of antigen presenting cells prevalent in the skin. These cells are responsible for initiating the adaptive immune response to an antigen [55].

2.3 Microneedles

Microneedles are needles reduced to the micron scale. The smallest needle available for hypodermic injection is 4 mm long [56], whereas microneedles range from 100 μm to 1.5 mm in length [57, 58]. The core concept of microneedles is that these small needles minimally penetrate past biological diffusion barriers for targeted drug delivery. Examples include: 1) passing the outer layer of the skin, the stratum corneum, for drug delivery to the skin [59], 2) passing the outer layer, the sclera, for drug delivery to the back of the eye [60], and passing cell membranes for drug delivery to cells [61].

Transcutaneous drug delivery was the first clinical application considered for microneedles [62, 63] and remains the predominant application of microneedles today [8, 9]. In pre-clinical and clinical studies, microneedles have delivered hormones [10, 11, 43], vaccines [64], nucleic acid chains [65], and other biopharmaceuticals to the skin.

There are generally four types of microneedles: 1) solid microneedles, 2) coated microneedles, 3) dissolving microneedles, and 4) hollow microneedles [8]. Figure 2.1 shows the method of drug delivery to the skin for each type of microneedle. Figure 2.2 shows an example image of each type of microneedle.

Microneedles have advantages over other transcutaneous drug delivery methods. The solid-state microneedles (solid, coated, and dissolving microneedles) are conceptually inexpensive and easy to use [8, 26] with limited dose variability, a concern with delivery via the mucosal route [66]. Hollow microneedles offer the highest degree of control over drug delivery rate for any transcutaneous method and may eliminate the need for drug reformulation [67].

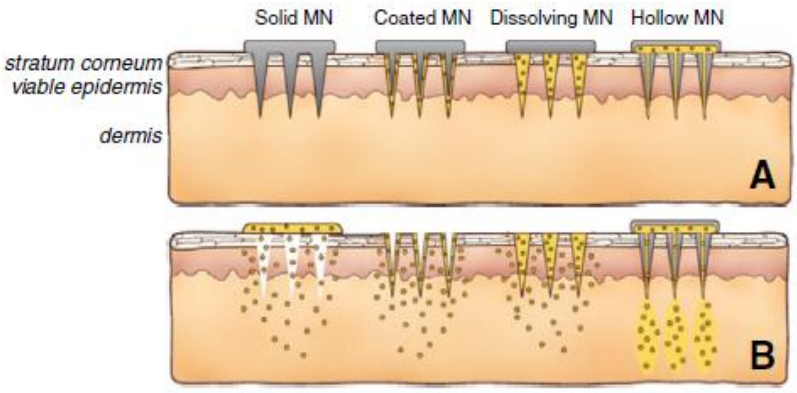

Figure 2.1. Methods of drug delivery to the skin using microneedles (MN). Microneedles insert into the skin (A) and then deliver drugs (B). Solid microneedles pretreat the skin, after which a topical formulation is applied that diffuses through the open pores. Coated microneedles deliver a coating of drugs that dissolves in the skin. Dissolving microneedles contain drugs in a composite matrix that dissolves in the skin, leaving no microneedle afterwards. Hollow microneedles inject fluid into the skin. [8]

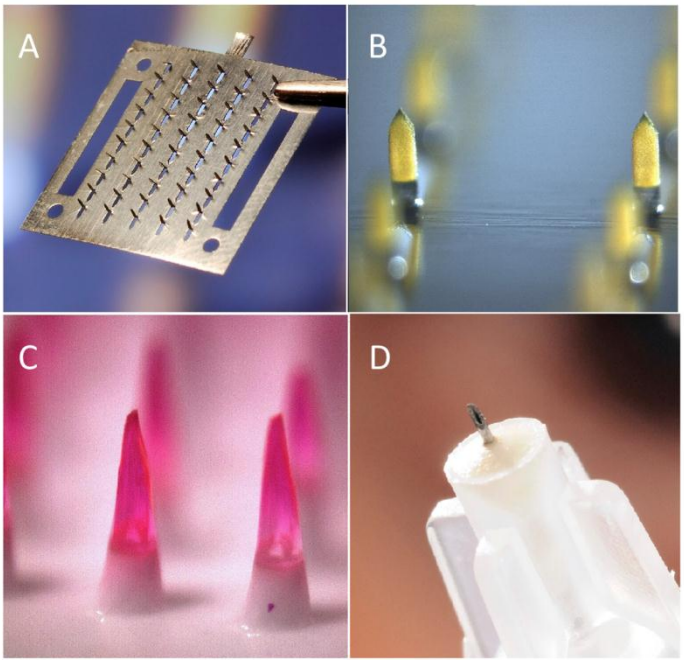

Figure 2.2. Example image for each type of microneedle: a) solid microneedles, each microneedle is 750 µm long (credit: Gary Meek, Georgia Tech), b) coated microneedles, each microneedle is 750 µm long (credit: Harvinder Gill, Georgia Tech), c) dissolving microneedles, each microneedle is 650 µm long (credit: Sean Sullivan, Georgia Tech), d) hollow microneedle, 850 µm long (credit: Gary Meek, Georgia Tech)

2.3.1 General Design Criteria for Microneedles

The different types of microneedles have similar design considerations. The choice of length depends on the target tissue and the drug delivery requirements, but microneedles are generally 1.5 mm or less in length for targeting purposes and pain minimization. Concerning needle number, solid, coated, and dissolving microneedles have a larger drug delivery capability in an array format (e.g., a 10x10 array of 100 microneedles) [8]. However, single hollow microneedles are often sufficient, as the amount delivered is not constrained by needle size or number [67].

Sharp tips are necessary to facilitate insertion into tissue [68-70]. This mandates some kind of taper in a microneedle's geometry, either from the base up as with conical and pyramidal geometries (Fig 2.2C) or from a higher-up point (Figure 2.2 A,B,&D). The geometry away from the tip matters for insertion depth, as slender microneedle designs [71] will insert deeper than tapered designs with low aspect ratios [72]. Material choice is constrained to biocompatible materials or generally-regarded-as-safe (GRAS) materials approvable by the FDA, e.g., for parenteral injection. Mechanical failure of microneedles is a concern given their small size and constraints on material choice. A common guideline in the literature for preventing mechanical failure of microneedles is that the insertion force for a particular microneedle must be much less than its critical buckling load [68, 70, 73], although microneedles can fail due to non-axial forces as well.

Microneedles can be inserted into skin manually, by applying pressure with a thumb, or automatically, with the help of a mechanical applicator. Applicators help with microneedle insertion by consistently applying the correct amount of force for insertion,

but they also add expense. Applicators that apply microneedles at high speed limit the skin deformation that occurs during manual microneedle administrations. A summary of microneedle applicators is available in [74].

2.3.1.1 Solid Microneedles

As shown in Figure 2.1, solid microneedles create pores in the skin for enhanced drug transport. The main function of a solid microneedle is to insert into the tissue with minimal pain and survive the process without breaking. Solid microneedles are typically made of stiff materials (materials with high elastic moduli: metals, silicon, ceramics) to maximize microneedle robustness while minimizing microneedle size [8]. Fabrication methods include laser cutting, injection molding, and various etching processes [8].

2.3.1.2 Coated Microneedles

Coated microneedles are essentially solid microneedles coated with a drug formulation so that upon insertion, the coating inserts with the microneedles and dissolves in the tissue. Co-localizing the drug onto microneedles limits the potential amount delivered, but simplifies administration compared to solid microneedles by eliminating the need for an external drug source [75]. Coating formulations often contain viscosity enhancers, surfactants and chemical stabilizers alongside drugs to enhance the amount coated, to obtain a uniform coating, and to protect the drugs' stability after drying [8]. Coating methods include dip coating [75], spray coating [76], and layer-by-layer coating [77]. Coated drugs in a solid state can have enhanced thermostability compared to drugs kept in a liquid state [78, 79]. The enhanced thermostability could obviate the need for a cold chain for vaccine distribution.

2.3.1.3 Dissolving Microneedles

Dissolving microneedles are an extension of coated microneedles where the entire microneedle dissolves in the skin as opposed to just a coating. The key advantage is that dissolving microneedles, after use, should not constitute sharps waste and pose little-to-no risk of needlestick injury because the needles have dissolved [80]. Drugs in dissolving microneedles can also have enhanced thermostability [81].

One drawback of dissolving microneedles is that the strongest available dissolving materials are approximately 100-times less stiff than non-dissolving materials used for solid microneedles. To resolve this issue, dissolving microneedles have broader bases compared to solid microneedles to increase the critical buckling load [68, 72]. These large, low-aspect-ratio microneedles have two complications: 1) typically, only half of the microneedle length inserts into tissue and 2) the larger microneedles take longer to dissolve in skin, e.g., > 5 minutes, depending on design. Separable "arrowhead" microneedles posted on metal shafts overcome these issues by increasing the functional microneedle length and depositing the dissolving arrowhead in the skin to dissolve [82]. Dissolving microneedles have been made out of natural and synthetic polymers using micromolding and drawing techniques [8].

2.3.1.4 Hollow Microneedles

Whereas other microneedle types rely on passive diffusion of drugs, hollow microneedles inject drugs using pressure-driven fluid flow, much like a hypodermic needle injection. The advantages of hollow microneedles compared to passive delivery methods are: 1) faster delivery; 2) greater control over delivery rate, especially in conjunction with an electric pump [67]; 3) larger delivery capability because the amount

delivered is not constrained by microneedle size; and, 4) no need for reformulation of drugs [67]. Reducing hollow needles to the micron scale has many challenges, though: 1) fabrication is inherently more difficult and costly compared to non-hollow microneedles, 2) the hollow interior reduces the critical failure load and makes hollow microneedles more fragile upon insertion, 3) integrating with a pressure source after fabrication adds additional complexity, 4) leaks are a concern because hollow microneedles do not insert very deep into tissue, and 5) the small lumens on hollow microneedles can become sealed by tissue requiring them to be offset from center [69, 83]. Researchers have made hollow microneedles out of glass, photolithographic materials, silicon, plastics, and metal [8]. Several techniques have been used to fabricate hollow microneedles: microelectromechanical systems (MEMS) techniques, laser machining, bevel grinding, hot embossing, injection molding, and electrodeposition onto sacrificial molds [8].

2.4 Insulin Delivery

2.4.1 Diabetes

Diabetes, also known as diabetes mellitus, is a chronic disease characterized by abnormally high concentrations of glucose in the blood [84]. Glucose accumulation in diabetes stems from problems with insulin production or insulin resistance. Insulin is a peptide hormone that regulates carbohydrate and fat metabolism. One critical function of insulin is to induce cells to uptake glucose. When this process fails, diabetes occurs.

Several types of diabetes exist. Type 1 diabetes mellitus results from destruction of a patient's insulin secreting cells (pancreatic islet β cells) with patients prone to a secondary fat metabolism disorder called ketoacidosis. The majority of type 1 diabetes

cases are caused by autoimmune destruction of the β cells (type 1A), but 10% of cases are idiopathic (type 1B). Approximately one million people in the United States have Type 1 diabetes. Type 2 diabetes results insulin resistance, reduced sensitivity of the cellular glucose uptake mechanism, combined with defects in insulin secretion. Type-2 diabetes accounts for 90-95% of diabetes cases in adults in the United States [85]. A small number of other diabetes cases result from pregnancy, genetic disorders, chemical toxicity, and other endocrine diseases.

2.4.2 Insulin Therapy for Type 1 Diabetic Patients

The insulin delivery work in this thesis focuses on insulin delivery for type 1 diabetic patients. Type 1 diabetic patients require insulin therapy, but the majority of type 2 diabetic patients do not [85]. Insulin therapy for type 1 diabetic patients has two components: bolus delivery and basal delivery [84]. Bolus insulin delivery is delivery of insulin over a short time frame to account for the expected rise in blood glucose after a meal. Basal delivery of insulin mimics natural delivery of a small amount of insulin to account for insulin needs in basal metabolic processes.

Different types of insulin are available with unique amino acid sequences and pharmacokinetic profiles. Regular human insulin mimics insulin naturally produced by human β cells and has a peak insulin concentration in the blood 2-3 hours after injection (see Figure 2.3). NPH insulin, an intermediate-acting insulin, has a slightly slower onset. Fast-acting insulins (lispro, aspart, and glulisine) have faster onset times than regular human insulin and provide improved glycemic control for bolus delivery [86]. Faster acting insulin analogs are in development to mimic the insulin release kinetics from β

cells [87]. The action of current insulin analogs is too slow to allow automatic control of blood glucose, so patients must plan each insulin injection and monitor their blood glucose afterwards. Ultra-fast-acting insulins could enable automatic control of blood glucose, also known as closed-loop insulin therapy [88].

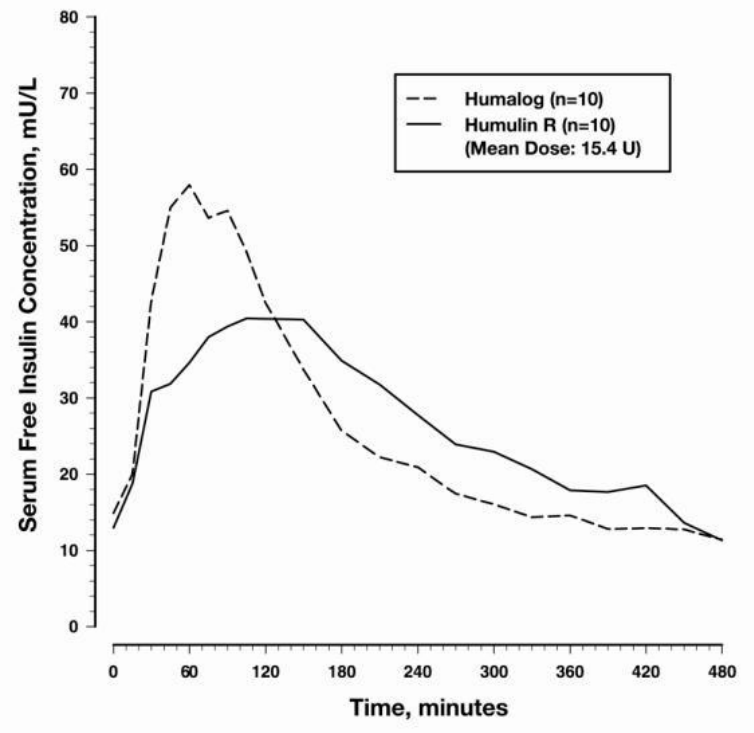

Figure 2.3. Time-action profiles of insulin lispro (Humalog) and regular human insulin (Humulin R), both administered subcutaneously. Insulin lispro is faster acting compared to regular human insulin (figure from Humalog package insert, Eli Lilly & Co.)

2.4.3 Approved Insulin Administration Methods

All current insulin formulations approved for use in the United States are administered subcutaneously by injection with a needle and syringe [84]. An inhalable

insulin formulation was available briefly but taken off the market due to poor sales [89]. Jet injection is available but not recommended for routine use [24].

Two common methods of administering insulin include: 1) a daily injection of a long-acting insulin with bolus injections of regular- or fast-acting insulin for each meal and 2) continuous subcutaneous insulin infusion (CSII) using a programmable electronic pump. CSII uses the same insulin formulation, often a fast-acting insulin, for continuous basal insulin infusion and programmed bolus infusions for each meal. The pumps administer insulin through a subcutaneous infusion catheter (SIC) which patients insert with a spring-loaded hypodermic needle.

2.5 Vaccine Delivery

2.5.1 Introduction to Vaccines

Vaccines provide protective immunity against infectious disease before a natural infection occurs [55]. In the United States, vaccines are available to protect against over 25 distinct viral and bacterial pathogens [90]. Different types of antigens are used as vaccines: attenuated live pathogens, inactivated pathogens, purified surface components of pathogens, toxins produced by pathogens, and toxins covalently bonded to other antigens. Vaccines are sometimes delivered with adjuvants, such as aluminum salts that improve immune responses through multiple mechanisms such as controlled release of an antigen and enhanced uptake by antigen-presenting cells [91].

Part of this dissertation focuses on seasonal influenza vaccine delivery. Seasonal influenza is a viral respiratory disease with sufficient antigenic drift to cause yearly epidemics in the fall and winter [92]. This differs from pandemic influenza outbreaks that result from large, infrequent antigenic shits. Most current influenza vaccines are

split vaccines, i.e., influenza viruses split into a mix of constituent parts, or subunit vaccines composed of purified influenza virus surface proteins [93]. These vaccines are injected either intramuscularly or intradermally. A live-attenuated influenza vaccine (LAIV) is available for nasal administration with approximately 10% market share overall [94] and a one-third market share in children [95]. Seasonal influenza vaccines contain three distinct antigens to account for multiple possible co-circulating strains [92]

Beginning in 2010, the Advisory Committee on Immunization Practices (ACIP) of the United States recommended universal seasonal influenza vaccination for all people above six months in age, expect for those contraindicated against the vaccine [25]. Children under nine years of age receiving their first seasonal influenza administration receive two doses. All others receive one dose. People who are moderately or severely ill are contraindicated against receiving the vaccine. Those who have life-threatening allergies, have had severe reactions to an influenza vaccine, or have had Guillain-Barré syndrome are expected to consult with a doctor before receiving a vaccine [96]. The seasonal influenza vaccination coverage (the percentage of recommended people who receive the seasonal influenza vaccine) in 2011-2012 was 45.5 ± 1.5% [5], well below targeted levels.

One vaccination concept important for this dissertation is vaccine efficacy. From [92], "vaccine efficacy is the percentage reduction in disease incidence attributable to vaccination." When the reduction in disease incidence is measured in a prospective randomized, controlled trial, the term vaccine efficacy is used. If the observation of disease reduction is retrospective, the term vaccine effectiveness is used.

The effectiveness of seasonal influenza vaccines is limited by vaccine composition and antigenic drift. Inactivated influenza vaccines lack immunogenicity [93, 97], and newer vaccines and formulations are expected to offer improved effectiveness. LAIV offers improved effectiveness in children, but evidence is mixed for adults [98]. Studies to assess the effectiveness of a high dose inactivated influenza vaccine are ongoing [99]. Intradermal injection offers equal efficacy at reduced and equal doses compared to intramuscularly injected influenza vaccines [100]. Newer skin immunization methods, including coated and dissolving microneedles, have not been evaluated for efficacy or effectiveness in humans. Efficacy improvements after microneedle immunization are hypothesized, based on animal data [64].

2.5.2 Approved Administration Methods

The majority of vaccines are administered intramuscularly or subcutaneously [101]. The intramuscular route is used for most inactivated vaccines including inactivated influenza vaccines. The subcutaneous route is used for certain live-attenuated vaccines: measles, mumps, rubella, and varicella-based vaccines.

In the United States, the FDA and ACIP have approved eight vaccines for alternative administration routes: oral rotavirus vaccine, oral adenovirus vaccine, oral typhoid vaccine, intranasal LAIV, intradermal inactivated influenza vaccine, intradermal Bacillus Calmette-Guérin (BCG) vaccine, intradermal rabies vaccine, and smallpox vaccine administered with a bifurcated needle [25, 90]. The WHO has prequalified two other vaccines for alternative administration in international use: oral polio vaccine and

oral cholera vaccine [102]. Several new methods for administering vaccines are in development, including microneedles [8, 47, 66, 103-106].

2.5.3 Vaccination Settings

In the United States, routine immunizations are given in multiple settings: traditional healthcare settings, pharmacies, mass vaccination sites, and patients' homes [7, 101]. The traditional healthcare settings (doctors' offices, clinics, and hospitals) are the primary location for all childhood and adult immunizations. The pharmacy setting is used for vaccinations in all 50 states, primarily for adult vaccinations [107]. Mass vaccination sites include work sites, town halls, and schools. Vaccination at home is only available for the oral typhoid vaccine in the United States. For influenza vaccination in 2012, 57% of vaccinations were given in traditional healthcare settings, 20% in pharmacies, and 23% at mass vaccination sites [5]. Other vaccination settings are used for hard-to-reach populations in developing countries or for non-routine immunizations to stop outbreaks or pandemics [108].

2.6 Behavioral Analysis with the Theory of Reasoned Action

This dissertation includes a behavioral analysis based on the theory of reasoned action to explore determinants of people's choice between a microneedle patch and an intramuscular injection for influenza vaccination. Icek Ajzen and Martin Fishbein introduced the theory of reasoned action in 1967 as a universal theoretical model for predicting behavior [109]. Their description of the model follows, and a schematic of the model is shown in Figure 2.4.

"According to the theory of reasoned action, a person's intention is a function of two basic determinants, one personal in nature, and the other reflecting social influence. The personal factor is the individual's positive or negative evaluation of performing the behavior; this factor is termed *attitude towards the behavior*. … The second determinant of intention is the person's perception of the social pressures put on him to perform or not perform the behavior in question. Since it deals with perceived perceptions, this factor is termed *subjective norm*. … Generally speaking, individuals will intend to perform a behavior when they evaluate it positively and when they believe that important others think they should perform it."

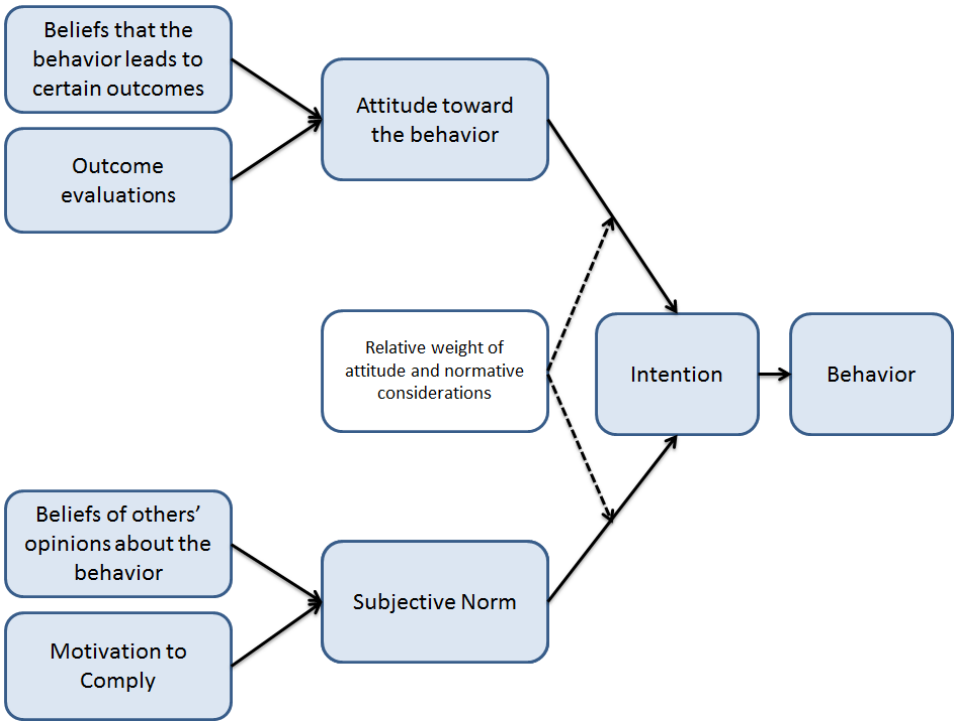

Figure 2.4. Schematic of the theory of reasoned action [109].

A meta-analysis of studies based on the theory of reasoned action illustrates the utility of this behavioral model. "Not only does the model appear to predict consumer intentions and behavior quite well, it also provides a relatively simple basis for identifying where and how to target consumers' behavioral change attempts" [110]. The

meta-analysis also found that while Fishbein and Ajzen intended to model only single behaviors, the theory performs well for modeling choices of behaviors. Examples of choices of behaviors would include whether to receive a vaccine and whether to choose a particular device for a vaccination.

Alternatives to the theory of reasoned action include the theory of planned behavior [111], a modification that includes perceived behavioral control as a factor affecting intent, and the health belief model [112], a predictive model of health behavior based on perceived susceptibility, severity, barriers, and benefits.

2.6.1 Methods for Generating and Analyzing Data for a Theory of Reasoned Action Model

This dissertation focuses on modeling intent using the theory of reasoned action. Modeling this case requires measurements of intent as well as measurements of the individual constructs [109].

A behavior needs to correspond to a specific action acting on a specific target within a given time and context. An example of a behavior is receiving an influenza vaccine within the next year at a patient's preferred location (e.g. doctor's office or pharmacy). Intent to perform that behavior can be measured in several ways: a yes/no-binary measure, a measure of percent likelihood to perform that behavior, or a scale based measure (e.g. from 0-10 likelihood). Differential intents, differences between likelihoods, can be used to model a choice between two behaviors.

The individual constructs, such as attitudes or subjective norms, are typically measured using scales consisting of a series of questions. The topics addressed in the constructs are difficult to assess with a single question. A scale provides a way to measure multiple aspects of a construct and condense it into a single measurement [113]. When multiple scales are measured using the same questionnaire, factor analysis can be performed to ascertain whether the scales are distinct from one another as factors and whether the scales have adequate internal consistency [114, 115]. Factor analysis produces orthogonal, independent scales that can serve as variables for modeling behavioral intent.

2.6.2 Example Behavioral Studies Concerning Vaccine Acceptance

Behavioral models have characterized factors affecting vaccine acceptance. Example studies include models for acceptance of influenza vaccines [116-119], human papillomavirus vaccines [115, 120-124], pneumococcal vaccines [125, 126], hepatitis B vaccines [127], and hypothetical vaccines against HIV or gonorrhea [128-130]. For influenza vaccination in adults, studies show that the attitude towards vaccination is a stronger predictor of vaccination acceptance than subjective norms.

2.7 Economic Analysis of Vaccination

New vaccines or vaccination devices may offer a variety of healthcare benefits such as prevention of disease or reduced administration costs. However, newer vaccines and devices add healthcare costs, especially when replacing existing off-patent, mass-produced alternatives [131]. A general research question for a vaccine-related economic analysis is whether the improved benefit is "worth" the increased cost. Answering this

question can help determine how a new vaccine or device should be priced [132]. The worth is subjective and depends on perspective, timeframe, and the health outcome considered.

A variety of perspectives are available for economic analyses of vaccination, and the choice of perspective affects which costs and benefits are included in the analysis [133]. For example, patients may prefer self-administering a vaccine because they do not have to wait to receive a vaccine. However, a healthcare payer (an agency providing health insurance or health benefits) is only concerned about the cost of delivering a service and would not include patient time in an economic analysis of a new device. For analyses presented to government agencies, the societal perspective, encompassing all possible costs and benefits, is the preferred perspective [131, 134]. The healthcare payer's perspective is a useful alternative for devices for adult vaccinations, since healthcare payers are likely the primary purchasers of the devices.

Because the benefits of a vaccine may not accrue until several years after vaccination (e.g. liver cancer prevention after hepatitis B vaccination), the timeframe and the method for valuing future costs and benefits are important considerations for an economic analysis [131, 133]. Most of the costs and benefits of influenza vaccination occur within one year, so the timeframe for economic analyses of influenza vaccinations typically use a 1-year timeframe with no discounting of future benefits [7].

Different types of economic analyses exist for different outcome measures [131, 133]. A cost benefit analysis (CBA) assesses whether the benefits (in units of currency) of a new vaccine or device outweigh the costs. The drawback of CBA is that it is

difficult to convert some vaccination benefits, like reduced deaths, to units of currency. A cost effectiveness analysis (CEA) assesses costs in terms of prevented health outcomes. CEA is useful for comparing two similar interventions that result in the same prevented health outcomes. Cost utility analysis assesses costs in terms of standardized health metrics such as quality adjusted life years (QALYs) or disability adjusted life years (DALYs). Cost utility analyses enable comparisons of disparate health interventions on equal terms, but QALYs and DALYs are subjectively measured and the measurement methodology is controversial [135, 136].

Economic analyses tend to be prospective and rely on assumptions and uncertain inputs. Even after well-done investigations, some additional information is needed to answer specific economic questions [133]. Economists use mathematical models to fill in the missing information. In a mathematical model, assumptions can be varied to see the effect on cost-effectiveness, and uncertain inputs can be sampled repeatedly from random distributions to generate confidence intervals and most-likely values for outputs. Some mathematical models can also be used to study the dynamics of disease transmission for different interventions [137]. Sensitivity analyses of mathematical models allow modelers to find what parameters affect the output most. If the output is significantly affected by an assumption of a measurable variable (e.g. patient waiting time for vaccination), this suggests further research in measuring that variable to reduce uncertainty for decision makers [131, 133].

2.7.1 Economic Analysis of Vaccination Devices

Economic analyses of vaccination devices have been performed to examine the tradeoff between increased device costs and several factors: improved safety compared to standard injections [138-140]; dose sparing due to alternative delivery routes [105, 140, 141]; reduced administration costs [105, 139], reduced waste [140, 142], improved effectiveness [143], and reduced number of doses needed [140]. Self-administration of vaccines may constitute a new way to reduce administration costs. In addition, a new factor to consider for devices is the ratio of patients who prefer self-administration to healthcare-worker-administration when both options may be available. This will affect the cost-effectiveness because the weighted-average administration cost will drop as more patients prefer self-administration.

CHAPTER 3

MOLDING PROCESS FOR HOLLOW METAL MICRONEEDLES

3.1 Introduction

Intradermal injection has pharmacological benefits over alternative injection methods for various different drug delivery scenarios. For vaccines, injection into the skin enables dose sparing and increased immunogenicity [29, 140]. For dermatological medicines, intradermal delivery targets drugs to the site of action in the skin with higher bioavailability than topical application [144]. Newer applications of intradermal injection include improved lymphatic uptake of etanercept to treat autoimmune diseases like rheumatoid arthritis [44] and expedited pharmacokinetics of insulin delivery to improve treatment of diabetes mellitus [10, 11].

Despite the pharmacological advantages, intradermal injections are not widely used. The standard Mantoux technique requires a specially trained healthcare provider to insert a hypodermic needle at a grazing angle above the skin surface into the skin, which is approximately 1 – 2 mm thick [145]. Even when performed by trained professionals, intradermal localization is unreliable [27].

To simplify intradermal delivery, we and others have fabricated hollow microneedles that target drug delivery into the skin with needles that are shorter than the thickness of the skin [69, 83, 146-154]. These devices are expected to reliably target injections into the skin, even by healthcare professionals without special training on their use [155]. Additionally, microneedles may improve patient acceptability compared to intradermal injections due to their small size and reduced pain [67, 156, 157].

Previous research provides four guidelines to optimize microneedle design. First, microneedles should have a sharp tip to reduce the force required for insertion into the skin [69, 158]. Second, microneedles with side-opened lumens, off-center from the tip, may reduce injection pressure by initiating flow away from where the skin is most compressed [69, 159, 160]. Third, microneedles need an appropriate combination of material strength and geometry to survive insertion into skin without mechanical failure [68, 69, 146, 158]. Fourth and finally, the fabrication process should rely only on inexpensive methods such as micromolding, ideally rejecting complicated two-piece molds or fragile demoldable cores. [150, 153, 161-164]. A microneedle meeting these criteria could easily insert into skin, inject at a moderate pressure to reduce patient pain, and compete on costs with needles and syringes.

In this chapter, we present a fabrication process based on sacrificial micromolding and selective electrodeposition meeting the design criteria listed above. In addition, we characterize the device's insertion into skin and report *in vitro* and *in vivo* delivery studies with pig and hairless guinea pig skin.

3.2 Device Fabrication

We fabricated microneedle devices using a seven-step process: (i) fabrication of the master structure with a laser-ablated cavity, (ii) creation of a micromold based on the master structure (iii) creation of replicas of the master structure using the micromold, (iv) sputtering a gold seed layer onto the replicas, (v) selective electrodeposition to form the hollow microneedle structure, followed by (vi) dissolving of the sacrificial replicate structure to release the hollow metal microneedle and (vii) integration of the microneedle

with a syringe-based pressure source for fluid delivery. Figure 3.1 shows a schematic of the fabrication process, described in detail below.

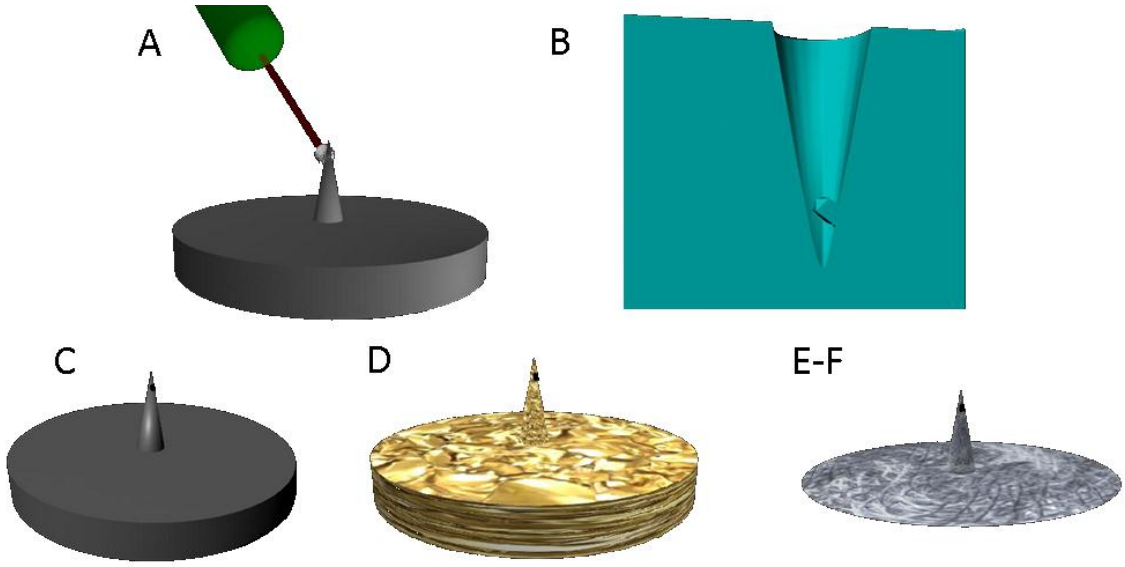

Figure 3.1 Fabrication sequence. (A) Fabrication of the master structure with a laser ablated cavity. (B) Creation of a micromold based on the master structure featuring a protruding pillar that will become the lumen exit hole in the final device. (C) Creation of a replica using the micromold. (D) Sputtering a gold seed layer onto the replica. (E) Electrodeposition of metal everywhere except the cavity, followed by dissolving of the sacrificial base material to release the metal microneedle.

3.2.1 Master Structure

We lathed a brass rod (VS Lathe, Harrison, West Yorkshire, UK) to produce a disk measuring 7 mm in diameter and 1 mm in thickness with a tapered cylinder (i.e., solid microneedle) measuring 1.1 mm tall, 225 µm in radius at the base, and 20 µm in radius at the tip, which was centered on top of the disk. A poly(lactic acid) (PLA) replica of the lathed structure was made using PDMS molding and melt casting (Park et al. 2005). To accomplish this, the lathed structure was attached to an aluminum dish (5 cm diameter, 1.5 cm deep) using double-stick tape, and covered with 15 g of

poly(dimethylsiloxane) (PDMS) (Sylgard 184, Dow Corning, Midland, MI). The PDMS was cured at 37°C for 24 h, and the PDMS mold was separated from the lathed structure by hand. PLA pellets (3051D, NatureWorks, Minnetonka, MN) were placed onto the PDMS mold, which was then placed in a 195°C vacuum oven. Vacuum was applied after the PLA had melted to degas the polymer and force it into the mold. After stopping the vacuum and cooling the samples, the PLA was separated from the mold by hand.

After molding, we laser-ablated a cavity in the PLA replica to create the feature that forms the lumen exit hole on hollow microneedles. The 70 µm x 70 µm cavity was ablated using a 248-nm excimer laser (Resonetics, Nashua, NH). The cavity was centered ~135 µm below the tip at a 45° angle relative to the base and was ~100 µm deep. The ablated microneedle served as the master structure and template for micromolding replicas.

3.2.2 Replicating the Master Structure

The PDMS molding and melt-casting procedures described above were used to create poly(lactic acid-co-glycolic acid) (PLGA) replicas of the master structure. The PLGA-filled mold was placed in a -20°C freezer for 5 min, taking advantage of differences in thermal expansion coefficients of PLGA and PDMS to facilitate demolding. Low molecular weight PLGA (7525 DLG 7A, Surmodics, Birmingham, AL) was used because it dissolves rapidly for sacrificial micromolding. Although the molds have a small, non-vertical core (Figure 3.1) that could complicate demolding, we did not experience problems because the PDMS molds were sufficiently flexible.

3.2.3 Seed Layer and Electrodeposition

We sputtered a gold seed layer onto each PLGA replica using an EMS 500 sputter coater (Electron Microscopy Sciences, Hatfield, PA). To monitor the variance in seed layer thickness, we measured the resistance across the diameter of each sample (edge-to-edge resistance) using a standard Ohmmeter. Thicker seed layers would have reduced edge-to-edge resistance.

We expect, because of masking effects, much less gold was deposited in the cavity compared to the rest of the solid microneedle structure. Therefore, the resistivity inside the cavity would be higher compared to the rest of the sample, enabling selective electrodeposition outside the cavity. As described previously in [165], for a sample with heterogeneous resistivity, the reduction of metal from the electroplating bath is more difficult on the area of high resistivity compared to the area of low resistivity.

We electrodeposited nickel onto individual solid microneedle devices using a Watts nickel bath (Watts Semi-Bright RTU, Technic Inc., Cranston, RI) and a constant-current power supply (E3611A, Hewlett Packard, Palo Alto, CA) with a range of 0-850 mA. The bath solution was stirred at 700 rpm, and the solution and anode were replaced after 36 h of electrodeposition.

To optimize operating conditions for deposition of metal onto the solid microneedle structure, without depositing over the cavity, we varied the seed layer thickness (as determined by edge-to-edge resistance) and the starting current of electrodeposition (Table 3.1). We tested seed layers with edge-to-edge resistances between ~10 Ω (thick seed layer) to ~44 Ω (thin seed layer). We varied the starting current from 0-9 mA.

Table 3.1 Effects of Starting Conditions on Electrodeposition Process Yield

Edge-to-Edge Resistance (Ω)[1]	**Starting Current (mA)[2]**	**Sample Size**	**Yield[3]**	**Mode of Electrodeposition Failure**
Low 10 ± 2	Medium $1.65 \leq I \leq 3.0$	3	0%	Immediate over-deposition[4]
Medium 20 ± 2	Low $1.0 \leq I \leq 1.65$	3	0%	No deposition
Medium 22 ± 6	Medium $1.65 < I \leq 3.0$	32	63%	Some over-deposition
Medium 18 ± 4	High $3.0 < I \leq 9.0$	5	20%	Over-deposition
High 44 ± 5	Medium $1.65 < I \leq 3.0$	9	11%	Under-deposition[5]

[1] Edge-to-edge electrical resistance (mean ± standard deviation) was used as a proxy measure for seed layer thickness.
[2] Electrodeposition was carried out at a starting current for 6 min at the beginning of the process.
[3] Yield: percent of devices with complete metal deposition on the mold surface and a patent lumen exit hole.
[4] Over-deposition: electrodeposition of metal covering the lumen exit hole.
[5] Under-deposition: electrodeposition of some metal, insufficient to cover the microneedle surface.

Thick seed layers led to immediate over-deposition of metal, filling the cavity and preventing hollow microneedle formation. With thick seed layers, presumably even the cavity had sufficiently low resistivity for electrodeposition. Thin seed layers suffered the opposite problem: insufficient metal deposition to cover the microneedle surface.

For intermediate seed layer thicknesses ($R \sim 20\ \Omega$), the deposition pattern changed depending on the starting current. Low currents ($I < 1.65$ mA) led to under-deposition, high currents ($I > 3.0$ mA) led to over deposition. Optimal conditions were found at a starting current of $1.65 < I < 3.0$ mA. The ability to switch between correct

deposition and over-deposition suggests selective electrodeposition is driven by seed layer resistivity differences in the cavity.

After 6 min at the starting current, we expedited the electrodeposition process by ramping up the current by 0.125 mA/min to a peak current of 4.75 mA for 2 h. Although the current increased, we did not lose the selectivity of the electrodeposition. We believe this was because as metal was deposited outside the cavity, the resistivity of the microneedle surface decreased and thereby increased the selectivity of metal deposition outside the cavity.

3.2.4 Dissolving the Sacrificial Replicate Structure

After this electrodeposition process, samples were inspected using bright-field microscopy (SZX 16, Olympus, Center Valley, PA). By shining a light on the backside of the sample, we determined which samples had maintained a patent lumen exit hole by seeing if the light passed through (Figure 3.2B). Samples passing inspection were suspended in an acetone bath stirred at 100 rpm for 1 h. The PLGA and the seed layer inside the cavity dissolved and disintegrated, releasing the metal hollow microneedle. We then electrodeposited a second time on these samples at 5.5 mA for 2 h to increase the metal thickness with no risk of over-deposition. The final metal thickness of five representative samples was 12.4 ± 1.4 μm, measured using brightfield microscopy.

3.2.5 Device Assembly

The fabricated microneedle was glued (Epoxy Plastic Bonder, Loctite, Rocky Hill, CT) to an acrylic device holder with a half-cylinder shape and aligned with a 3 mm diameter through-hole. The flat edge of the acrylic was glued using cyanoacrylate glue (Super Glue Gel, Loctite, Rocky Hill, CT) onto a nylon female luer lock cap (McMaster-

Carr, Atlanta, GA) which also had an aligned 3 mm diameter through-hole. The finished assembly was mounted onto a syringe.

3.2.6 Final Device Imaging

Images of fabricated hollow microneedles are shown in Figure 3.2. Figure 3.2A shows a hollow microneedle after the fabrication process. Figure 3.2B shows light passing through the lumen exit hole. Metal can also be seen inside the needle in Figure 3.2B. This short, tubular vestige from the cavity reveals the boundary of selective electrodeposition. . Figure 3.2C shows a PDMS mold used for the micromolding-based fabrication. Figure 3.2D is an image of a microneedle assembled onto a luer hub for connection to a syringe.

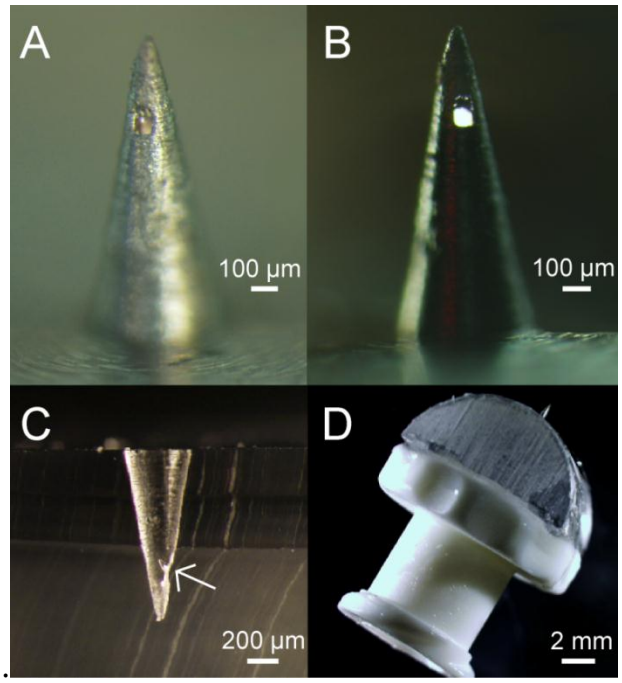

Figure 3.2 Optical microscopy images of the microneedle device. (A) Hollow microneedle imaged with direct lighting. (B) Hollow microneedle imaged with light shining through the backside, which illuminates the lumen exit hole of the microneedle. (C) Cross-section of the PDMS micromold with arrow pointing to demoldable core that forms the lumen exit hole of the microneedle. (D) Device assembly used for injections with a hollow microneedle glued to a curved acrylic surface mounted on a luer lock.

3.3 Force Displacement Measurement

We followed a method described previously [166] to characterize the axial failure force of the microneedles. Briefly, the needles were pressed against a steel plate at a constant speed until a discontinuity was observed in the force versus displacement graph. The force at the onset of the discontinuity was the measured failure force.

3.3.1 Experimental Results

The axial failure force for the hollow microneedles was 0.74 ± 0.11 N (average ± standard deviation, n =5 replicate measurements). A representative force-displacement curve is shown in Figure 3.3. Visual observation showed the mode of failure was telescopic collapse of the needles similar to seen previously with hollow metal microneedle [158]. The predicted force required to insert a needle with this tip geometry into human skin is 0.086 N [158]. The measured failure force is close to an order of magnitude greater than the predicted insertion force, which provides a large margin of safety.

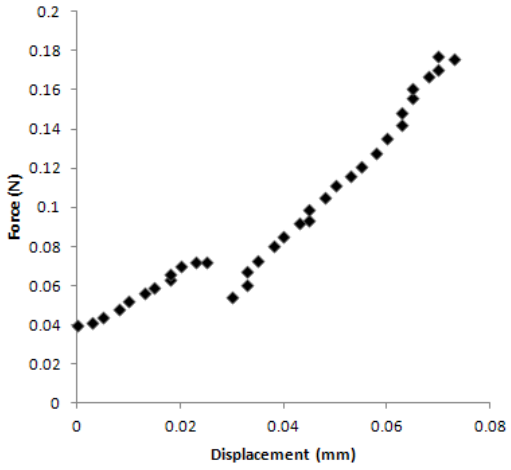

Figure 3.3 Representative force-displacement curve for a microneedle pressed against a rigid surface. A discontinuity representing microneedle failure is seen at 0.025 mm displacement with a peak force of 0.072 N.

3.4 Insertion and Injection into Pig Skin *In Vitro*

3.4.1 Insertion into Pig Skin

To validate the prediction that our microneedles should be able to be inserted into skin without mechanical failure, we pressed microneedles into pig skin *in vitro*, and then stained the insertion sites with gentian violet (Humco, Texarkana, TX), which selectively stains sites of microneedle penetration and not intact skin [156]. The microneedles were mounted onto a 3 ml syringe and applied to the skin by hand. As shown in Figure 3.4A, gentian violet staining confirmed the hollow microneedles were able to penetrate skin. We made four microneedle insertion attempts in this way each using a different microneedle, and all of them provided effective skin penetration. Additional successful microneedle insertions accompanied by fluid injection are described below.

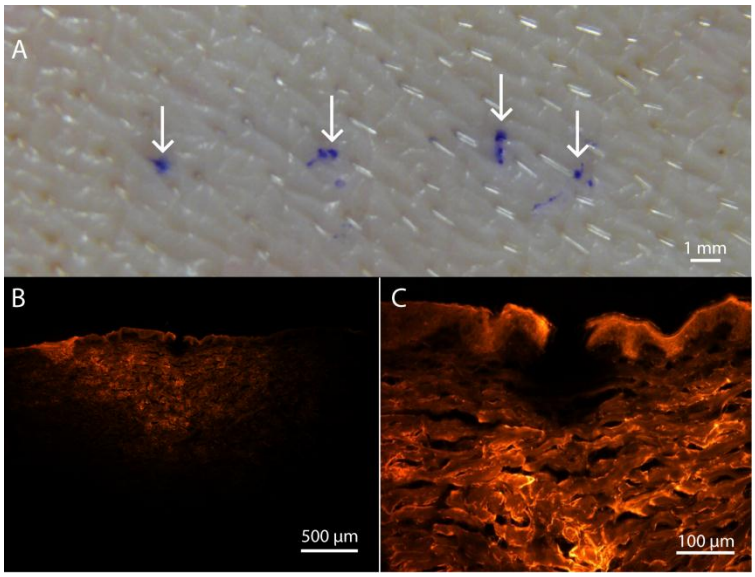

Figure 3.4 Microneedle injection into pig skin *in vitro*. (A) Photograph of skin surface after insertion of four microneedles followed by gentian violet staining (arrows indicate insertion sites). The stain demonstrates penetration of the microneedles into skin. (B) Fluorescence microscopy image of a skin cross-section after injection of red-fluorescent sulforhodamine B using a microneedle. (C) Magnified view showing the site of needle insertion into skin.

3.4.2 Injection into Pig Skin

We also verified the microneedles could inject fluid into skin. Using microneedles mounted onto 3 ml syringes, we applied thumb pressure for 20 s to inject 0.1 ml of a 1 mM solution of a fluorescent dye, sulforhodamine B (Sigma-Aldrich, St. Louis, MO), in phosphate-buffered saline (PBS) (Sigma-Aldrich). After injection, 1 cm x 1 cm pieces of skin were cut out, sectioned, embedded in Optimal Cutting Temperature compound (Sakura Finetek, Torrance, CA), and frozen on dry ice. The skin was cut into 10 µm sections using a Microm cryostat (Thermo Fisher, Walldorf, Germany). Skin sections, excited by a BH2-RFL-T3 light source (Olympus, Center Valley, PA), were photographed using a SZX16 microscope and DP71 camera (Olympus) to examine the distribution of sulforhodamine B in the skin.

Figure 3.4B shows a representative histological cross-section of the sulfrohodamine B delivery confirming these microneedles can deliver fluid into skin. The needle insertion site is shown at greater magnification in Figure 3.4C. We examined three skin sites after injection in this way, each using a different microneedle, which resulted in intradermal injection.

3.5 Insertion and Injection into Hairless Guinea Pig Skin *In Vivo*

We tested insertion and injection using microneedles *in vivo* on a hairless guinea pig (Charles River Laboratory, Wilmington, MA) with approval from the Georgia Tech Institutional Animal Care and Use Committee (IACUC). The guinea pig was anesthetized using isofluorane gas in oxygen and sustained in anesthesia using a nose cone. The microneedle was manually inserted into the skin on the back of the guinea pig,

and pressure was applied to the syringe plunger by hand. We delivered approximately 50 – 75 µL of a 1 mM solution of sulforhodamine B in PBS. We measured the peak pressure using a digital manometer (Model 220-95, Netech, Farmingdale, NY). After delivery, we applied gauze to the delivery site for 10 s and then placed the gauze in 4 mL of PBS for 30 min. The PBS solution was analyzed using a fluorometer (Spectramax Gemini XS, Molecular Devices, Sunnyvale, CA) to determine the amount of dye leaked onto the skin surface. At the end of the experiment, the guinea pig was euthanized using carbon dioxide and 1 cm x 1 cm pieces of skin and subcutaneous tissue were cut into 14 µm-thick sections as frozen sections and imaged as described above. Images were split into red, green, and blue channels using Image J. The fraction of dye delivered to the skin was calculated as the red intensity in the epidermis and dermis divided by the red intensity of the whole tissue section.

3.5.1 Experimental Results

Figure 3.5 shows a representative photographic image and histological cross-section of an injection site after microneedle injection into a guinea pig. The peak pressure for intradermal injection with microneedles was 16.4 ± 2.6 psig (average ± standard deviation, n = 3). Using the image analysis technique described above, the mean fraction of dye delivered to the skin is 90 ± 7%(n = 4; two from guinea pig, two from pig *in vivo* – data not shown).

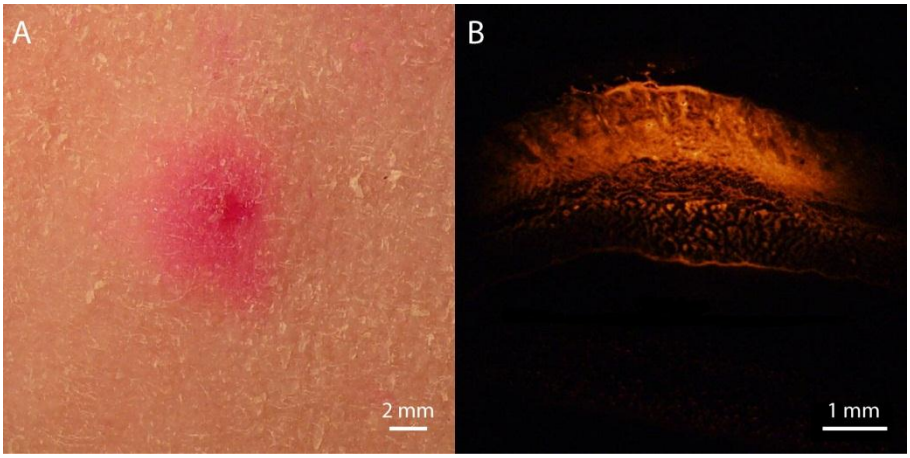

Figure 3.5 Microneedle injection into hairless guinea pig skin *in vivo*. (A) Photograph of the skin surface on the back of the animal showing injection of red-colored sulforhodamine B into the skin. (B) Fluorescence microscopy image of a skin cross-section after injection of red-fluorescent sulforhodamine B using a microneedle. On average, 90% of the dye was localized in the skin, which demonstrates effective targeting to the skin.

3.6 Discussion

We fabricated and tested hollow microneedles with the following advantageous features: (i) sharp tips, (ii) lumen exit holes located on the side of needles just below the tips, (iii) metal as the material of construction, and (iv) a micromolding-based fabrication process.

Human skin thickness varies across the body, but is generally 1 – 2 mm thick [167]. Here, we used microneedles measuring 1.1 mm in length to target injection into the skin by pressing the needle perpendicularly against the skin surface. This method should improve upon the conventional Mantoux technique that is unreliable even for trained experts.

The choice of fabricating a single, conical microneedle with a 20-25° taper was based on the observation that similarly shaped glass microneedles fabricated by pulled-glass micropipette techniques could insert into skin and deliver up to 1 mL of fluid in

human subjects [67]. The needles we fabricated were similarly able to insert into skin and inject fluid without leaks. The ratio of microneedle failure force to predicted skin insertion force of approximately 9 provides a large safety margin, allowing reliable insertion without fracture. This large safety margin was enabled by reducing insertion force by having a sharp needle tip and increasing needle strength by using metal as the material of construction.

Micromolding of hollow microneedles is challenging. For this reason, most previous studies have used direct fabrication methods: high precision etching, grinding, or laser ablation in the production stage. [54, 69, 146, 149, 168]. These methods are less attractive for inexpensive mass production because they require the use of sophisticated fabrication equipment often in a high-cost cleanroom environment to make each device. Direct fabrication of master structures followed by indirect molding-based replication should be much less expensive and enable the large-scale mass production needed for disposable needles. Among molding methods in the published literature, most have lumen exits at the microneedle tip which reduces sharpness and may impede fluid flow into tissue [83, 150, 154, 169]. Others have made molded hollow microneedles with side-opening lumen exit holes, but the molds were complex: either two-piece, aligned molds [163] or molds with seemingly fragile demoldable cores [147].

We introduced a fabrication method that lends itself to simpler mass production and produces microneedles with a sharp tip and side-opening lumen exit hole. After laser fabrication of a master structure, the master structure was repeatedly used to produce female micromolds. These micromolds were in turn used to make replicate structures employed as male micromolds for electrodeposition to produce metal microneedles.

While this approach involves an additional step to make the male micromolds compared to prior methods, it is an improvement because (i) it uses high-throughput molding methods to produce the microneedle devices and employs the lower-throughput laser etching process only to make the re-usable master structures and (ii) is able to mold metal microneedles with improved geometry that enable insertion and injection into skin.

The lumen exit hole was created by using a laser-generated divot in the master structure, which appeared to inhibit seed layer deposition and later electrodepositon. The divot in the master structure became a protruding core in the intermediate replicate mold, which might be expected to introduce complications in molding, especially during demolding. However, PDMS as the mold material provided sufficient flexibility and allowed reliable demolding with damaging the mold or the demolded structure.

We needed a balance to deposit metal onto the surface of the male mold, but not deposit metal within the cavity forming the lumen exit hole. A thin seed layer or a high starting current both led to over-deposition that covered the cavity. A medium seed layer thickness and a moderate starting current minimized over-deposition and enabled fabrication of suitable structures with reasonable yields. Further optimization of the microneedle geometry and electrodeposition process could improve yield.

Overall, this fabrication process produces metal hollow microneedles with simple, one-piece molds. Using an excimer laser to define the master geometry allowed precise positioning of the microneedle hole, and the PDMS-based molds were reproducible and reusable even with the angled pillar. The selective electrodeposition process may have other uses in microfabrication. Potential uses include depositing metal up to a certain

distance/elevation, avoiding deposition in high-aspect-ratio holes, or bonding two conducting materials with different resistivities as in [165].

CHAPTER 4

INSULIN DELIVERY USING MICRONEEDLES TO TYPE 1 DIABETIC CHILDREN AND ADOLESCENTS

4.1 Introduction

For children, adolescents, and adults with Type 1 diabetes, the most important goals are to improve compliance with diabetes management and prevent the long-term complications due to hyperglycemia. To address these needs, we evaluated the use of a single, hollow microneedle, less than 1 mm in length, to administer rapid-acting Lispro insulin to children and adolescents with Type 1 diabetes. We expect patient compliance to improve due to the microscopic length of the microneedle because of a reduction in pain and apprehension as compared to subcutaneous administration. We also expect the faster onset and offset of insulin pharmacokinetics due insulin delivery to the skin should improve closed-loop insulin therapy by being more responsive to changes in blood glucose levels.

Omission of insulin injections due to fear of needles is common in children and adolescents [3, 28, 170, 171]. Twenty-five percent of children and adolescents admit to omitting or underdosing injections due to needle phobia or anxiety [172, 173]. Fear and omission of injections correlate with worse HbA1C levels for children [1-3], leading to more frequent hospitalization and higher cost [174-176].

Improved needle designs may improve compliance with insulin therapy. Pen devices, shorter needles, and injection ports have provided small compliance

improvements in adults and children [177-179]. Small reductions in needle size have been helpful, however, microneedles are up to an order of magnitude shorter, and may be even more effective at reducing pain and apprehension. Typical fine needles for subcutaneous injection are 4-8 mm in length and 0.2-0.3 mm in diameter. In contrast, microneedles are typically less than 1 mm in length and 0.1 mm in diameter [8, 180]. In blinded trials, microneedles are less painful than hypodermic needle injections, and the reduction in pain is proportional to the reduction in needle length [156, 181]. In addition to patient reports of microneedles being less painful than subcutaneous injections, physicians have also agreed that microneedles would be less painful and provide a beneficial option for patients on insulin therapy [157]. These findings suggest that microneedles can improve compliance in children and adults because of reduced needle pain and apprehension.

Microneedles may also enable closed-loop therapy by reducing the lag time for onset and offset of insulin action. Closed-loop therapy is currently limited largely by long lag times associated with insulin delivery and glucose measurements, necessitating advanced algorithms to make accurate predictions of required insulin doses [88, 182]. These lag times do not exist in the normal pancreas, which is how a properly functioning pancreas can maintain tight control over blood glucose levels. Microneedles may enable closed-loop therapy by reducing the lag time for insulin onset and offset, thereby allowing closed-loop therapy to more rapidly respond to changes in blood glucose levels and ease the requirement to predict insulin delivery needs. Studies in adults have shown that intradermal infusion of insulin using microneedles leads to much faster uptake and subsequent clearance of insulin lispro and regular human insulin compared to

subcutaneous delivery. This is thought to be due to the enhanced venous and lymphatic access in the skin compared to the subcutaneous space [10, 11, 41, 42, 44].

Thus, microneedles offer two major routes to improved management of Type 1 diabetes: (i) improving compliance, especially in children and adolescents with needle phobia and anxiety, and (ii) accelerating insulin pharmacokinetics to enable more responsive closed-loop therapy. Although data on intradermal insulin delivery exist for adults [10, 183], it is unknown if microneedles reduce pain in children and adolescents for insulin administration or any other application. It is also unknown if rapid uptake of insulin after intradermal injection will occur in children and adolescents. We believe this is the first study of microneedles in a pediatric population for any application (although two pediatric participants were included as part of a larger population studied by our team [10]).

We conducted this trial in a pediatric population to test the hypotheses that delivery of lispro insulin using a microneedle results in (i) less needle-insertion and infusion pain and (ii) faster pharmacokinetics compared to traditional subcutaneous insulin delivery.

4.2 Research Design and Methods

Sixteen participants were recruited from patients followed in the Diabetes clinic at the Emory Children's Center Division of Pediatric Endocrinology. Subjects were 10-18 years of age, had Type 1 diabetes for at least 2 years, were using a conventional insulin pump for at least the past year, and had a BMI below the 85^{th} percentile (see Table 4.1). Subjects were excluded if they had type 2 diabetes, acanthosis nigricans, a major organ disease, an insulin requirement > 150 U per day, or a cognitive impairment (more than

two grades behind age-appropriate grade). Female subjects were excluded if they were pregnant or nursing. On the day of the study, all subjects were healthy, afebrile, and had a blood glucose level between 100 and 200 mg/dL.

This repeated-measures study involved microneedle and subcutaneous administration of insulin on separate days. The order of procedures was randomized using spreadsheet software. No investigators were aware of the order until the day of the study. Eight out of sixteen patients had microneedle administration first. No changes were made to the study design during the course of the study. This study was approved by the Emory University Institutional Review Board.

Study visits took place in examination rooms at the Emory Children's Center (Atlanta, GA). Subjects fasted the night before the study starting at 10 PM, arrived at the study site at 7 AM, and had an intravenous catheter inserted in the antecubital fossa. After intravenous catheter placement, a 10 mL initial blood specimen was collected, after which, insulin Lispro (U50) was administered by either the microneedle or subcutaneous route using a syringe pump set at a flow rate of 1.0 mL/min. The insulin dose was based on the subject's insulin-to-carbohydrate ratio for a 75-gram carbohydrate meal. Insulin dosing ranged from 10 – 20 U (i.e., 200 – 400 microliters of a U50 insulin Lispro solution).

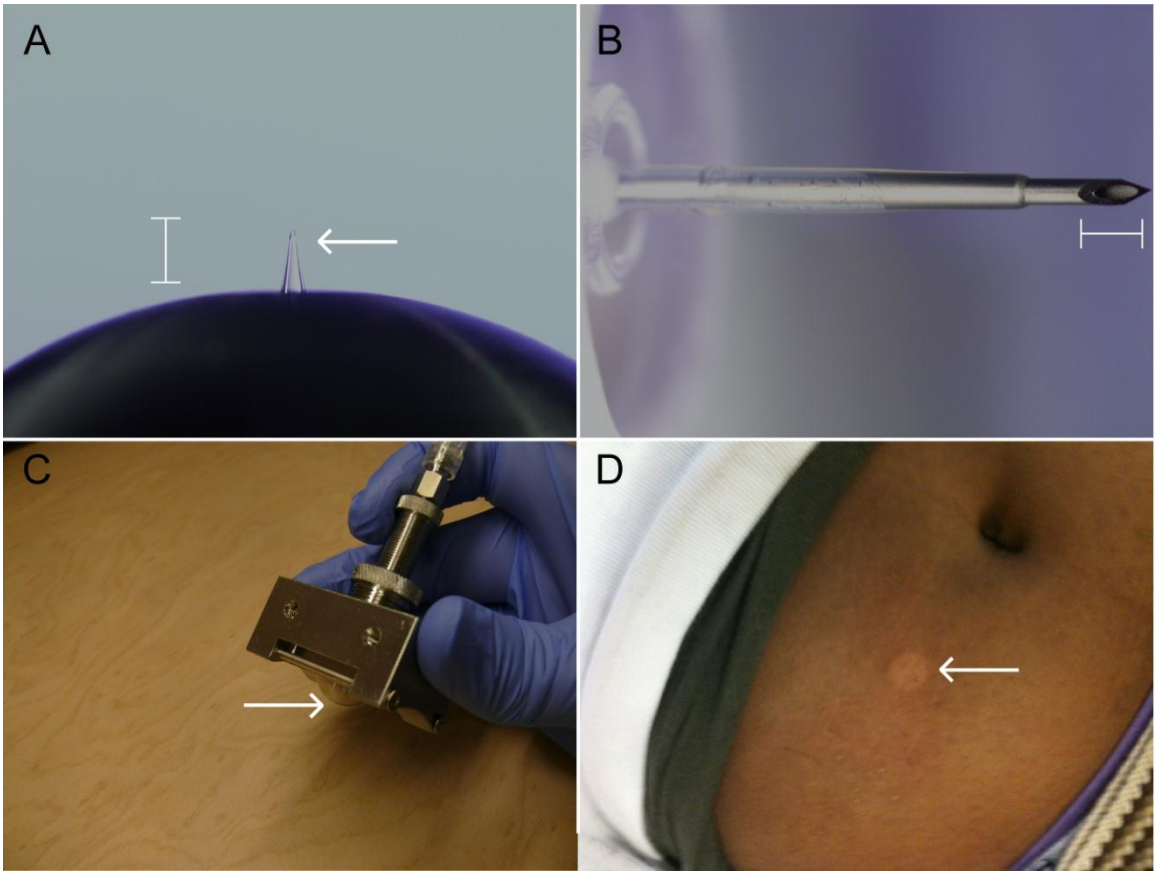

Figure 4.1 Microneedle used for intradermal insulin delivery. (A) Magnified view of a microneedle (see arrow) mounted on a curved holder. Scale bar is 1 mm. (B) Magnified view of a hypodermic needle used to insert a subcutaneous insulin catheter. Scale bar is 1 mm. (C) Device to hold and insert microneedles into skin. Arrow shows location where microneedle protrudes from the device. (D) Characteristic intradermal bleb (see arrow) on the abdomen of a participant after microneedle delivery.

For microneedle administration, the microneedle holder was placed on the skin, after which the microneedle was inserted into the skin to a microneedle length of 1.1 mm and then retracted to a final microneedle length of 0.9 mm prior to infusion. Figure 1A and Figure 1B compare a microneedle and a subcutaneous needle and catheter at the same magnification. Microneedles were made at Cartika Medical (Maple Grove, MN) and mounted in a microneedle holder (Figure 1C) described previously [41]. An investigator placed the microneedle in the microneedle holder and inserted the

microneedle into the skin in a rotating, drilling manner to minimize skin deflection during microneedle insertion, as described previously [10]. After infusion, the microneedle was retracted slowly (15 s) and a characteristic intradermal bleb was observed (Figure 1D). For subcutaneous administration, a 9-mm MiniMed infusion set (Medtronic, Minneapolis, MN) was used and inserted with the manufacturer's spring-loaded inserter by an investigator. All insulin delivery injections were performed on the subject's abdomen.

After insulin administration, subjects immediately ate a standard 75-gram carbohydrate meal. The meal was identical for all subjects on both study days. Blood was collected from the intravenous catheter every 15 min for 2 h and then every 30 min for an additional 2 h. Data collection was stopped if subjects developed hypoglycemia (blood glucose < 60 mg/dl) or symptomatic hyperglycemia as measured by a hand-held glucometer. Blood was collected in EDTA-containing tubes and then centrifuged, separated, and frozen immediately after collection. The frozen plasma specimens were assayed at the Emory Children's Center for glucose concentration (Glucose Colometric Assay Kit, Cayman Chemical, Ann Arbor, MI) and free Lispro insulin concentration (LisPro Insulin RIA, Millipore, Billerica, MA).

Outcome measures included pain measurements, plasma insulin concentration vs. time, and plasma glucose concentration vs. time. Pain was measured after insulin administration using a 100-mm slider as a visual analog scale (VAS). An investigator blinded to the administration method measured subjects' insertion and infusion pain.

All statistical analyses compared microneedle and subcutaneous administration within subjects. Sample size was determined based on the number of subjects needed to

achieve statistically significant differences in the area under the insulin curve (AUC), pain, and time to peak insulin concentration (t_{max}). Thirteen subjects were needed to detect a difference of 15 min with a standard deviation of 15 in t_{max} at a power of 90% and an alpha of 0.05. Only seven subjects were needed to detect a difference of 15 mm with a standard deviation of 10 in pain with insertion at a power of 90% and an alpha of 0.05. Therefore, given the possibility of up to a 20% dropout or loss-to-follow-up rate, we enrolled sixteen subjects.

Insertion and infusion pain were compared with paired t-tests. A pharmacokinetic model with first-order insulin absorption and elimination was fit to all participants' insulin curves [42]. t_{max}, offset time (time to return to half the peak concentration, $t_{1/2}$), AUC were derived from the pharmacokinetic fit. Time values were compared using paired t-tests. AUC and the pharmacokinetic coefficients from the model were compared using ratio paired t-tests. One post-hoc test examined the significance of the correlation between microneedle dose and t_{max}.

4.3 Results and Discussion

Sixteen subjects enrolled in this study and were seen between February 2009 and January 2012. Four participants' data sets were excluded from the analysis. One subject had an elevated insulin level at time 0 that was likely due to a bolus infusion for mild hyperglycemia that the subject did not inform the investigators of. Three others received incomplete doses of insulin due to a mechanical issue with the microneedle holder. Three subjects developed hypoglycemia at 3 h after both methods of insulin administration. No other clinically significant events occurred. The final analysis includes 12 subjects for 3 h. Participant demographics are shown in Table 4.1.

Table 4.1. Participant Demographics

Sample size	Number enrolled	n = 16
	Number included in analysis	n = 12
Gender	Male	n = 6 (50%)
	Female	n = 6 (50%)
Age	11 – 12	n = 3 (25%)
	13 – 14	n = 1 (8%)
	15 – 16	n = 6 (50%)
	17 – 18	n = 2 (17%)
Race / Ethnicity	Caucasian / White	n = 8 (67%)
	African American / Black	n = 4 (33%)
HbA1C average	7.0 – 7.4 %	n = 6 (50%)
	7.5 – 7.9%	n = 4 (33%)
	8.0 – 8.4%	n = 2 (17%)
BMI	17 – 19	n = 3 (25%)
	20 – 24	n = 6 (50%)
	24 – 29	n = 3 (25%)

Initially, pain was assessed by evaluating the VAS score after insertion of microneedles and subcutaneous catheters and by infusion of insulin solution. Microneedle insertion was significantly less painful than subcutaneous catheter insertion (Figure 2, $\Delta VAS = -9.9$, $p = 0.005$). Ten of the twelve subjects rated microneedle insertion as less painful than catheter insertion. Pain was also assessed after insulin infusion. There was no significant difference between pain experienced by subjects after infusion of insulin using the microneedle or subcutaneous catheter delivery (Figure 2, $\Delta VAS = 13.2$, $p > 0.05$).

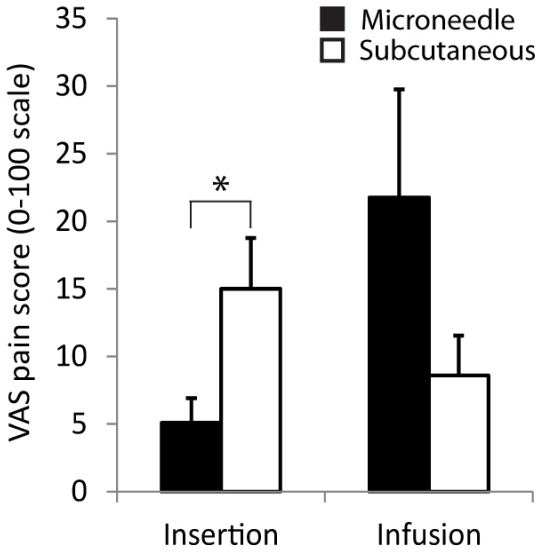

Figure 4.2 Pain of microneedle and subcutaneous insulin administration. Participants scored pain associated with insertion into skin and associated with insulin solution infusion using microneedles and subcutaneous catheters. The data represent average values from 12 participants. Error bars represent the standard error of the mean.
* = significant at $p = 0.005$.

Microneedle administration resulted in a faster onset and offset of insulin action (Figure 3A). The average time to peak insulin concentration (onset time) was 30 ± 2 min after microneedle administration and 52 ± 4 min after subcutaneous infusion (Figure 4A). The onset time for microneedle delivery was 22 min faster, a reduction of more than 40% ($p = 0.0004$). The offset time was also significantly faster for microneedle administration by 34 min, a reduction of 24% ($p = 0.017$).

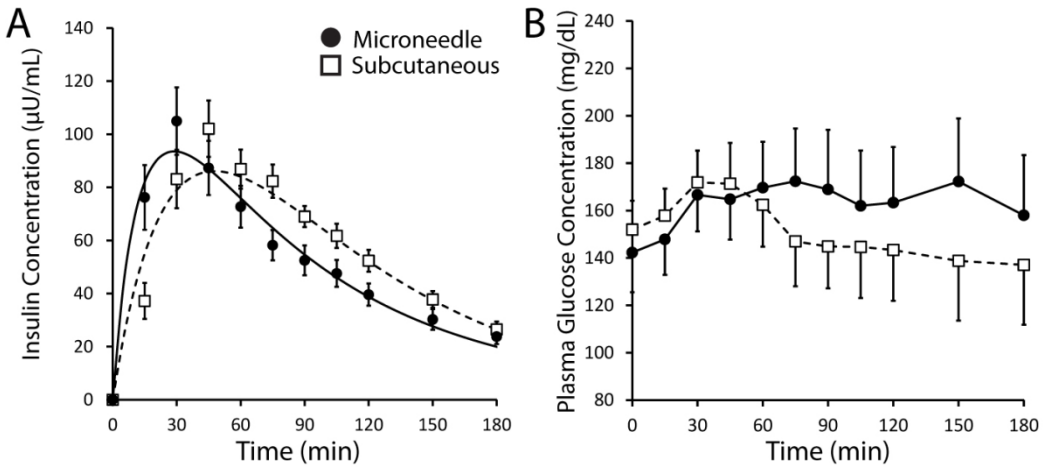

Figure 4.3 Pharmacokinetics and pharmacodynamics of insulin delivery. (A) Average insulin concentration vs. time. (B) Plasma glucose concentration vs. time. Black circles = microneedle, black squares = subcutaneous. The data points represent average values from 12 participants. Error bars represent the standard error of the mean. The lines represent the average of the fits from the pharmacokinetic model: solid line = microneedle, dashed line = subcutaneous.

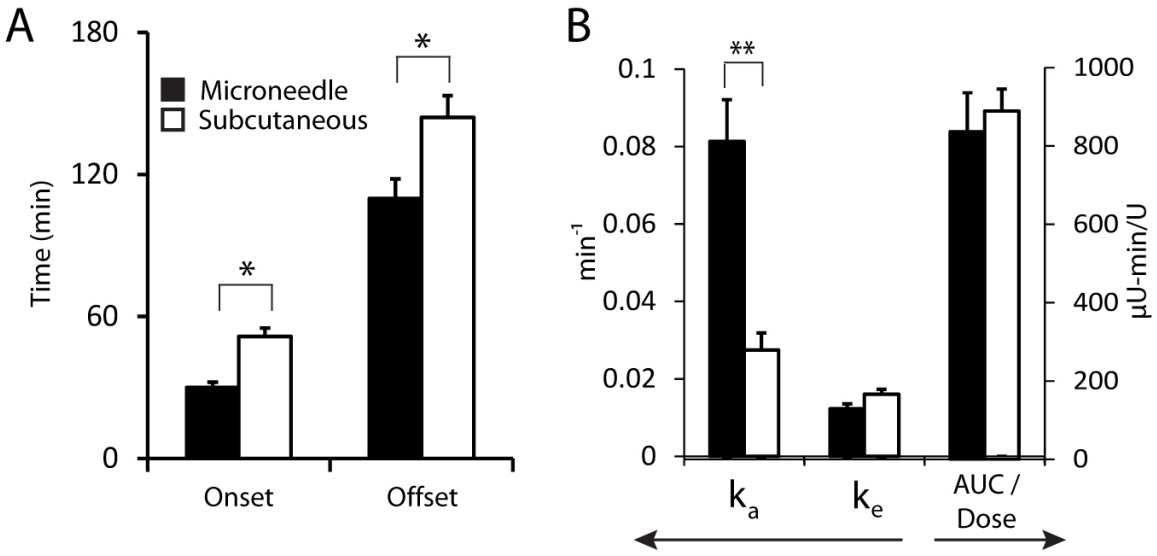

Figure 4.4 Pharmacokinetic analysis of intradermal insulin delivery using microneedles. (A) Insulin onset time for microneedle (black bars) and subcutaneous (white bars) administration. (B) Parameters derived from the pharmacokinetic model after delivery of insulin using microneedles and subcutaneous infusion: insulin absorption coefficient (k_a) insulin elimination coefficient (k_e) and area under the insulin curve normalized by dose delivered (AUC/dose). The data represent average values from 12 participants. Error bars represent the standard error of the mean. * = significant at $p < 0.05$. ** = significant at $p = 0.0002$.

Pharmacokinetic modeling provides additional measures for comparison between the two delivery methods. The absorption coefficient was 3.7 times higher for microneedle administration (Figure 4B), consistent with the faster onset and offset times. The elimination coefficients were not significantly different (Figure 4B), indicating that once insulin reaches the bloodstream by either route, it is eliminated at the same rate. Finally, the AUC values were statistically indistinguishable between the two groups (Figure 4B). This is expected because the same amount of insulin was delivered by each route to each subject.

The pharmacodynamic response to insulin delivery after ingestion of a standard carbohydrate meal is shown in Figure 3B. After insulin administration by either route, plasma glucose level rose over the course of 30 min, which is expected after consuming

food. After microneedle delivery, glucose levels plateaued for the remainder of the study, whereas glucose levels decreased and returned to baseline in subjects receiving subcutaneous insulin.

This study tested the hypothesis that insulin delivery using microneedles causes less insertion and infusion pain in children with type-1 diabetes compared to conventional subcutaneous injection. Consistent with this hypothesis, we found that children rated insertion of microneedles as significantly less painful than introduction of a subcutaneous catheter. This is consistent with earlier studies of microneedles in adults [10, 156, 181] suggesting that microneedles may be useful for increasing insulin therapy compliance among children as well. This first study of microneedles in children also suggests use of microneedles for therapy in other pediatric indications for improved compliance. Insulin delivery using microneedles did not cause less infusion pain compared to the subcutaneous route. This is also consistent with earlier results [10, 42]. Some strategies to reduce infusion pain associated with microneedles include: lowering the flow rate, adding a spreading enzyme such as hyaluronidase, and increasing the size of the needle lumen to reduce the hydrostatic pressure [67]. Adopting these strategies may also reduce the likelihood of incomplete delivery with microneedles, which possibly occurred in our studies.

This study also tested the hypothesis that intradermal insulin delivery using microneedles leads to faster onset and offset of insulin pharmacokinetics compared to subcutaneous injection. Consistent with this hypothesis, we found that insulin administered with microneedles had significantly faster onset and offset of insulin action.

Both the onset and offset times were reduced. This is consistent with previous pharmacokinetic studies of microneedles in adults [10, 11].

We believe that the reason for accelerated pharmacokinetics after insulin administration using microneedles involves targeting insulin delivery to the skin, which is enabled by the small size of microneedles. Skin delivery may target the rich capillary bed found in the superficial dermis, explaining the rapid insulin onset. An alternate explanation is that insulin injection into skin leads to rapid insulin uptake via lymphatic drainage [44, 184]. This study does not provide further insight into mechanism but does demonstrate accelerated pharmacokinetics with intradermal delivery.

In this study, post-prandial glucose for the microneedle group initially increased by approximately 30 mg/dl and then plateaued. A similar pharmacodynamic profile occurred in a study of adults all receiving an insulin dose at 0.125 U/kg [42] and in the 30% -under-dosed arm of a multi-arm study where participants received individualized doses [183]. The optimally-dosed arm in the latter study, in contrast, had a small but significant improvement in post-prandial glucose levels for intradermal insulin delivery. This suggests that optimizing dose for intradermal delivery is important to controlling post-prandial glucose levels, and that participants in our study may have been under-dosed overall. Microneedle administration to the skin, with faster onset and reduced persistence of insulin, may be better suited to rapidly responsive, closed-loop insulin therapy rather than standard subcutaneous insulin injections. The fast onset and fast offset could reduce problems with closed-loop insulin delivery caused by slow insulin response and persistence of insulin in the blood, such as the rapid rise of post-prandial glucose and the glucose peak above acceptable ranges caused by slow onset of insulin

action and the hypoglycemia caused by slow offset of insulin [88, 182]. Compared to implants being studied for closed-loop therapy that deliver intraperitoneal insulin with rapid pharmacokinetics [4], microneedles may offer a minimally invasive and more widely acceptable approach.

To improve closed-loop control, further reductions in onset and offset time with microneedles could be explored. For example, additional statistical analysis of our data showed that the time to peak insulin concentration was significantly shorter in subjects receiving smaller insulin doses delivered with the microneedle ($p < 0.02$). A similar correlation was previously observed and modeled for subcutaneous insulin delivery too [185]. Given this observation, it is possible that switching from a single injection on the order of 100 µl (as done in this study) to multiple injections on the order of, for example, 10 µl (using, for example, an array of multiple microneedles) could accelerate the uptake of intradermal insulin. Other strategies exist for accelerating the pharmacokinetics of insulin uptake including injecting hyaluronidase [186], warming the injection site [187], and creating a more soluble insulin formulation [87]. Combining these methods with microneedles may produce a synergistic effect resulting in ultra-fast insulin uptake. Limitations of our study include small sample size with limited demographic distribution and examination of only single insulin injections. To generalize our results toward possible future clinical adoption, additional studies are needed with larger pediatric trials and application of multiple insulin doses over multiple days to better assess dose-to-dose reproducibility of pharmacokinetics and pharmacodynamics, as well as long-term safety, tolerability, compliance and glycemic control. While the microneedles used in this study were made of borosilicate glass, we have also made similar microneedles out of medical-

grade stainless steel [188] and believe these metal microneedles will be more suitable for future use in clinical practice.

CHAPTER 5

USABILITY, ACCEPTABILITY, AND COST-EFFECTIVENESS OF MICRONEEDLE PATCHES FOR SELF-VACCINATION AGAINST INFLUENZA

5.1 Introduction

Vaccines save lives, but vaccine administration needs improvement to increase coverage and reduce costs [189]. This is especially true for influenza vaccination, where coverage rates are well below recommended levels and vaccination costs exceed $2 billion per year [5, 7, 190]. Self-administered vaccines, made possible by recent advances in vaccine delivery technology such as microneedle patches, are expected to improve coverage and cost-effectiveness [47]. This study, using microneedle patches to simulate influenza vaccination, provides the first measurements of the impact of self-vaccination on coverage and cost-effectiveness.

The United States (US) recommends universal seasonal influenza vaccination but achieves just 45% coverage [5], leaving influenza as the 8th leading cause of death [191] with over 200,000 hospitalizations [6] and 3,000-49,000 deaths [192] each year. Coverage levels for other voluntary vaccines are as low as 14% [193]. These low coverage levels are caused in part by fear of needles and inconvenience for patients. Needle phobia causes at least 7-8% of vaccination non-compliance [194] , and inconvenience ranks as high as second as a reason for skipping influenza vaccination [195-197].

Although increased vaccination coverage reduces morbidity and mortality, it further increases vaccination costs. Thus, reduced vaccination cost is of great interest. The key barriers to improving cost-effectiveness of influenza vaccination are administration costs and patient time, which outweigh the cost of the vaccine itself 3.3 to 1 [7].

To improve coverage and cost-effectiveness, new vaccination methods need to overcome needle-phobia, inconvenience, and high costs of vaccine administration. We propose to achieve this improvement using microneedle patches for high-throughput vaccination by healthcare personnel or self-vaccination by patients themselves. Microneedles patches contain arrays of needles measuring hundreds of microns in length that target vaccine delivery to the skin in a simple, minimally invasive and painless way [8, 74]. Microneedles can be manufactured at low cost by leveraging tools of the microelectronics industry as solid needles made of metal or polymer that encapsulate or are coated with vaccine that is released by dissolution in the skin within minutes.

Other approaches proposed for simplified or self-vaccination include intranasal, sublingual, oral, inhaled, edible and transcutaneous vaccines [198]. Only one vaccine, oral typhoid, has been approved for self-administration, with an estimated 3 million vaccine series administered per year worldwide [12, 199]. Microneedle patches, however, are especially attractive because they are compatible with live, inactivated and subunit vaccines [200, 201], administer a consistent dose [66, 202], offer the possibility of thermostability [79, 203] and can be manufactured inexpensively [8]. Additionally, microneedle-based influenza vaccines are expected to be well accepted by practitioners and the general public [57, 204, 205] and, as an intradermal delivery method, are more

immunogenic, as shown by non-inferiority at 20-60% of the full dose [54, 206] and superior seroprotection for older adults at full dose [207]. Microneedle patches have been used previously for self-administration of parathyroid hormone, but usability was not specifically studied [43]. A hollow microneedle device for self-injection of influenza vaccines was usable, safe, and effective [208]. In that study, in which the majority of participants worked in a healthcare setting, those who experienced self-vaccination were more likely to accept it in the future.

Despite interest in self-administration of influenza vaccines, there are no published data on self-administration of microneedle patches or on the effect of self-vaccination on vaccination coverage and cost-effectiveness. We therefore conducted a study on the usability, acceptability, and cost-effectiveness of microneedle patches for influenza vaccination to test three central hypotheses. First, participants can correctly apply microneedle patches with minimal training. Second, intent to vaccinate increases if a self-administered microneedle patch is offered to participants. Third, self-vaccination against influenza results in less cost to improve coverage compared to traditional vaccination.

Three minor objectives were included to compare the pain of microneedle patch administration and intramuscular (IM) injection, evaluate a hypothetical high-effectiveness patch for acceptability and cost-effectiveness, and weigh the factors affecting microneedle uptake using a theory of reasoned action framework [109].

5.2 Methods

5.2.1 Microneedle Patch Fabrication

We bent etched, stainless steel microneedles 90° out-of-plane, cut adhesive foam backing and liner material (TM9942, MacTac, Stow, OH) with an arbor press, and cut polyacetal packing pieces with a CO_2 laser. Parts were assembled with double sided adhesive (1522, 3M, Minneapolis, MN) and sent for ethylene oxide cycles. These placebo microneedles were designed to mimic coated microneedles [209].

5.2.2 Study Approval and Participant Recruiting

This study was approved by the Georgia Institute of Technology Institute Review Board, and informed consent was obtained from all participants. We used a venue sampling method [210, 211] to obtain a high response rate. Eligible participants were healthy, non-pregnant adults with no diseased skin, no pain perception problems, and no allergies to compounds used in the study. Seventy participants were recruited from Atlanta, GA between 9/11 and 5/12. Participant demographics are in Table 5.1. Because males and participants with a household income less than $20,000 were overrepresented, we modified our venue list during the study. Participants ranged from 18-62 years old, and all participants were naïve concerning microneedle use.

Table 5.1 Participant Demographics

Trait	Value	Count	Percentage of Sample (n=70)
Gender	Male	43	61%
	Female	27	39%
Race/Ethnicity	White	30	43%
	African American / Black	32	46%
	Other	8	11%
Education	High school or less	28	40%
	Associates degree	10	14%
	College degree or more	32	46%
Age	18-19	4	6%
	20-29	25	36%
	30-39	13	19%
	40-49	16	23%
	50-59	11	16%
	60+	1	1%
Income	Less than $20,000	18	26%
	$20,000 - $40,000	17	24%
	More than $40,000	26	37%
	Student, no income	8	11%

5.2.3 Experimental Procedures

Participants experienced three un-blinded procedures in a random order: self-administration of three microneedle patches, investigator-administration of a microneedle patch, and investigator administration of 0.5 mL IM saline injection. Patches were applied with thumb pressure. A computer application set the random order, with 46% of participants (32/70) experiencing self-administration before investigator-administration. Participants had written instructions for self-administration, and were told to push harder if their administration attempts failed. Pain ratings were collected using a visual analog scale (VAS). All participant input was collected using a computer to minimize reactivity.

5.2.4 Skin Staining to Measure Usability

After each patch application, we applied a dye to evaluate usability. We hypothesized more than 85% of people could correctly administer a microneedle patch, defined as >85% of needles observed as inserted. Expecting 67 of 70 participants to administer the patch correctly, we had 83% power for this hypothesis. Gentian violet and fluorescein were applied to different skin tones at investigator's discretion and imaged using a standard camera. Gentian violet 1% (Humco, Texarkana, TX) was pooled for 1 min, dabbed with gauze, and cleaned with alcohol after 5 min. Fluorescein 10% (Akorn, Lake Forest, IL) was diluted to 1% in saline, applied lightly with a cotton swab, dabbed with gauze, and cleaned with alcohol after 5 s. Fluorescein stains were imaged under blue LED light with blue glass as an excitation filter and a photography filter for emission (5558, Tiffen, Hauppauge, NY). Usability was quantified by counting insertion sites, uniformly and linearly narrowing the color balance on some images. Uncertain points, such as those covered by blood, were excluded from counts.

5.2.5 Questionnaires to Measure Acceptability

An adaptive survey solicited participants' willingness-to-pay for vaccination options. Participants chose between an IM injection, no vaccination at all, or a microneedle patch option. The IM injection had a fixed price (the lower of $25 or the participant's current out-of-pocket cost). The patch price changed according to a binary search algorithm. Each binary search had four steps starting at a random price, bounded between the IM price ± $20. This search conservatively underestimated willingness-to-pay. If the participant was willing to pay more than $20 above the IM price, they gave an open-ended willingness-to-pay. We asked about three vaccine patch options: self-administration at home, self-administration with a healthcare worker nearby, and healthcare-worker-administration. We repeated these measurements for a hypothetical "high-protection" patch offering a 50% smaller chance in getting influenza after vaccination.

To answer what factors drove acceptance of microneedle patches, we included a questionnaire with constructs borrowed from the theory of reasoned action [109]. The analysis method is described in Appendix A.

5.2.6 Quantitative Economic Analysis to Assess Cost-Effectiveness

A probabilistic Monte Carlo model explored two potential effects of introducing a microneedle patch: reduced influenza illness and improved cost-effectiveness. The model only included adults age 18-64. Probability distributions for influenza illness, vaccine efficacy, and vaccination costs were taken from an existing publication (Table 5.2 and 5.3) [7]. The cost of self-administration was set equal to the cost of pharmacy administration, which already excluded administrator labor costs. Uncertainty in

microneedle patch uptake was generated by bootstrapping this study's acceptability data. Each outcome measure was calculated 100,000 times to estimate uncertainty.

Table 5.2 Inputs of the Economic Model – Illness Parameters and Costs

Distributions for Predicting Coverage and Illness Rates	**Distribution**	**Parameters**	**Source**
Stated preference data	bootstrapped	n/a	current study
Standard vaccine effectiveness	triangular[1]	a = 0.3, b = 0.9. c = 0.69	1
Influenza attack rate	triangular[1]	a = 0.014, b = 0.155, c = .0066	1
Current coverage level	normal	μ = .455, σ = 0.0114	2
Current choices of vaccination setting[2]	normal	μ's from chart, $\sigma = 0.05*\mu$	2
Costs – 2010 USD	**Distribution**	**Parameters**	**Source**
Traditional setting – dose cost	constant[3]	11.57	1
Traditional setting – administration cost	triangular[1]	a = 16.31, b = 54.32, c = 24.35	1
Pharmacy – dose cost	triangular[1]	a = 8.46, b = 11.21, c = 9.37	1
Pharmacy – administration cost[4]	triangular[1]	a = 4.31, b = 10.93, c = 5.14	1
Mass vaccination – dose cost	triangular[1]	a = 3.47, b = 12.53, c = 11.11	1
Mass vaccination – administration cost	triangular[1]	a = 8.77, b = 10.65, c = 10.24	1

1 – Triangle distributions were created using data available from the original reference with a = minimum, b = maximum, and c = mode.
2 –The traditional setting included "doctor's office", "clinic or health center", and "hospital." The pharmacy setting included "pharmacy or store" and "other medically-related place." The mass-vaccination setting included "workplace", "health department", "school", and "other non-medical place". A standard deviation of 5% times the mean was applied to all values from the original chart. The sum of the fractions representing each setting is renormalized to 1.0.
3 – No distribution was provided in the original reference
4 – Self-administration costs were set equal to pharmacy costs, which did not include administrator labor.

Sources:
1. Prosser LA, *et al.* (2008) Non-traditional settings for influenza vaccination of adults: costs and cost effectiveness. *Pharmacoeconomics* 26(2):163-178.
2. http://www.cdc.gov/flu/professionals/vaccination/nfs-survey-march2012.htm

Table 5.3 Inputs of the Economic Model – Disease Rates

Population Statistics	**Distribution**	**Parameters**	**Source**
Number, age 18-49	constant	135 million	1
Number, age 50-64	constant	59 million	1
% at high risk, 18-49	constant	17.8%	2
% at high risk, 50-64	constant	34.1%	3
Illness Statistics[1,2,3]	**Distribution**	**Parameters**	**Source**
Hospitalization rate, 18-49, healthy	triangular[4]	a = 9.8, b = 40, c = 14	4
Hospitalization rate, 18-49, high-risk	triangular[4]	5 times rate of healthy individuals	4
Hospitalization rate, 50-64, healthy	triangular[4]	a = 27, b = 232, c = 70	4
Hospitalization rate, 50-64, high-risk	triangular[4]	5 times rate of healthy individuals	4
Death rate, 18-49, healthy	triangular[4]	a = 0.016, b = 0.68, c = 0.22	4
Death rate, 18-49, high-risk	triangular[4]	a = 0.4, b = 3.88, c = 1.11	4
Death rate, 50-64, healthy	triangular[4]	a = 0, b = 13.59, c = 3.31	4
Death rate, 50-64, high-risk	triangular[4]	a = 0, b = 67.8, c = 16.55	4

1 – Case rate was determined using the influenza attack rate in Table S.3
2 – The population basis for these rates was the total population minus those protected by the vaccine
3 – All rates given as events per 100,000
4 – Triangle distributions were created using data available from the original reference with a = minimum, b = maximum, and c = mode.

Sources:

1. United States 2010 census
2. http://www.cdc.gov/flu/professionals/vaccination/pdf/influenza_vaccine_target_populations.pdf
3. http://www.cdc.gov/flu/professionals/vaccination/pdf/targetpopchart.pdf
4. Prosser LA, *et al.* (2008) Non-traditional settings for influenza vaccination of adults: costs and cost effectiveness. *Pharmacoeconomics* 26(2):163-178.

5.3 Results

5.3.1 Usability

We first determined if participants could correctly apply microneedle patches with minimal training. After reviewing an instruction sheet with four sentences of instruction accompanied by four pictures, subjects self-administered placebo microneedle patches three times, had a placebo microneedle patch administered by study personnel and received an IM injection of saline in randomized order.

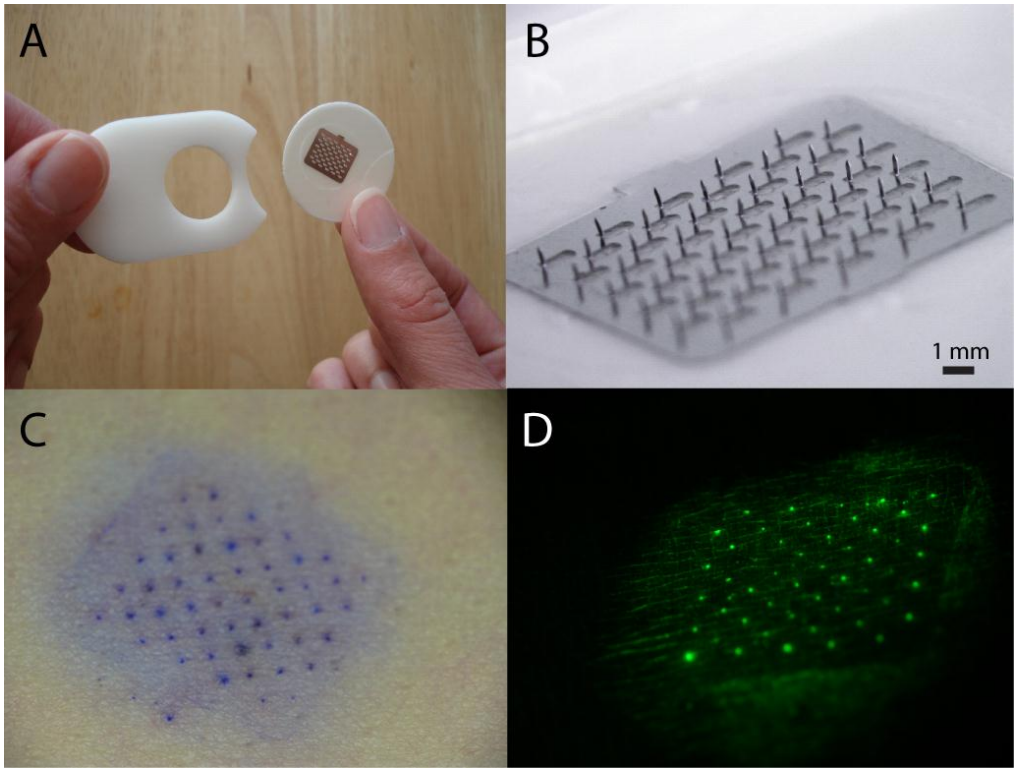

Figure 5.1 A placebo microneedle patch and examples of skin stains applied to evaluate usability. (A) A 12x10 mm microneedle array on a 30 mm foam adhesive patch with a polyacetal liner that protects the microneedles in packaging. (B) The microneedle array under magnification. Each microneedle is 750 μm long. (C) Gentian violet skin stain. (D) Fluorescein skin stain.

Given three attempts at self-administration, 95% of participants had a successful attempt (63/66, CI: 88-98%, Wilson score interval), confirming our hypothesis that participants can correctly apply microneedle patches with minimal training. Fig. 2 charts the usability data for each attempt and the best attempt out of three for self-administration, as well as usability of microneedles administered by study personnel. Usability data for four participants were unavailable. Three had unquantifiable fluorescein stains; one skipped self-administration when feeling light headed after IM injection, and was removed from analysis.

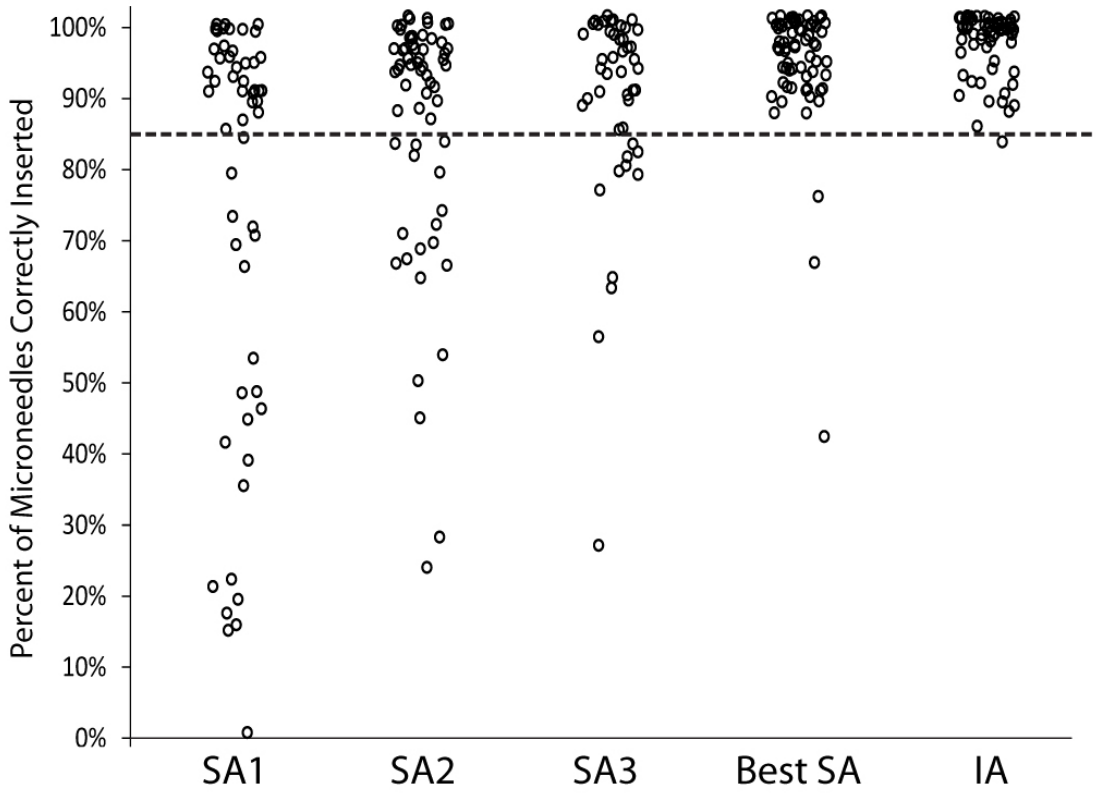

Figure 5.2 Usability of microneedles versus administration attempt. Attempts one through three are participant self-administrations. Best attempt is the highest percent administered from the three attempts. The control is investigator administration. The dashed line indicates our criterion for a successful patch application. A random jitter was applied to separate overlapping points (±1% on the y-axis).

Participants improved their success rate with each attempt. The success rate for the first attempt was 58% (38/63, CI: 45-69%), the second was 70% (45/64, CI: 57-81%), and the third was 75% (44/59, CI: 61-85%). Practice with self-administration led to significantly improved insertion rates ($p = 0.003$, $n = 57$, Friedman's rank test). Experiencing investigator administration before self-administration (due to randomization) did not significantly effect microneedle patch usability for participants' first attempts ($n = 63$, $p = 0.09$, Mann-Whitney U test). All procedures were well tolerated with only very mild, transient erythema. One unrelated adverse event occurred, a viral pneumonia case four days after the study.

5.3.2 Acceptability

We next assessed whether more participants would express intent to be vaccinated against influenza if offered a microneedle patch. As a baseline data point, 45% of participants expressed intent to be vaccinated against influenza during the coming year given currently available vaccination methods. This agrees well with 46% influenza vaccination coverage reported in the US in 2012 [5]. When participants were offered vaccination using a microneedle patch administered by a healthcare professional in addition to vaccination by IM injection, intent to vaccinate increased to 62%, which corresponds to a suggested coverage improvement of 17% (12/69, CI: 10-28%) (Fig. 3A). Among those expressing intent to be vaccinated, 60% preferred the microneedle patch and 40% preferred IM injection.

We next offered the option to self-vaccinate using a microneedle patch, either at home or in the presence of a healthcare worker. Given these additional options, intent to vaccinate increased to 64%, representing a 19% (13/69, CI: 11-30%) increase in intent to

vaccinate compared to vaccination offered only by currently available methods (Fig. 3B). Among those expressing intent to be vaccinated, 45% preferred to self-administer the microneedle patch at home, 9% preferred to self-administer the microneedle patch in the presence of a healthcare worker, 16% preferred to have a healthcare worker administer the microneedle patch, and 30% preferred IM injection.

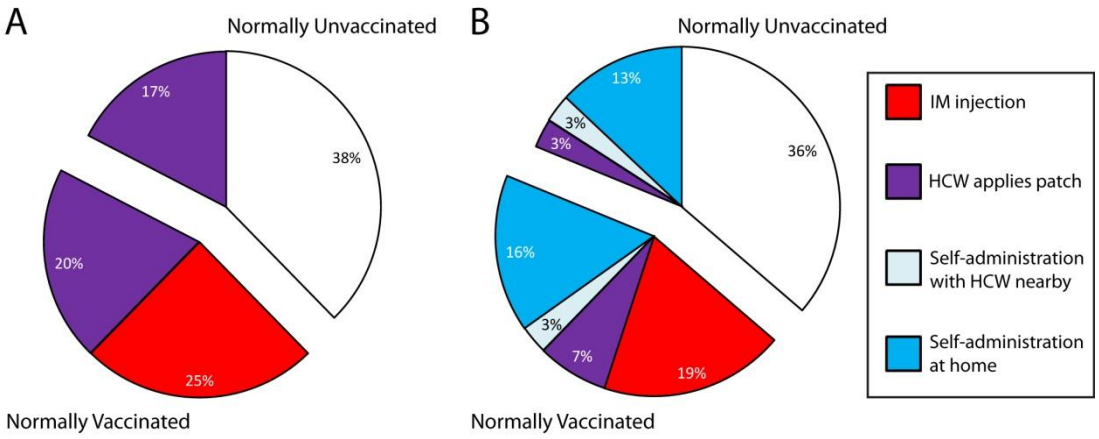

Figure 5.3. Acceptability of microneedles and self-vaccination. For a patch priced equally to an intramuscular injection, participant preference for four vaccination options is shown: i) intramuscular injection, ii) healthcare worker (HCW) applies a patch, iii) self-administration of a patch with a healthcare worker nearby iv) self-administration of a patch at home. The white slice shows participants who would remain unvaccinated. (A) Acceptability of microneedle patches without self-vaccination. (B) Acceptability of microneedle patches with self-vaccination.

These data show that offering a microneedle patch dramatically increased intent to vaccinate. Although most participants preferred to self-vaccinate, there was only a small incremental increase in intent to vaccinate due to offering self-vaccination. These data confirm out hypothesis that intent to vaccinate increases if a self-administered microneedle patch is offered to participants.

To better understand the role of economics in the choices that participants made, we evaluated intent to vaccinate as a function of the price differential between out-of-pocket cost to the participant for IM injection versus the microneedle patch. As shown in Figure 5.4, reducing the price of the microneedle patch could further increase intent to vaccinate by about 1% per dollar decrease, with an increasing fraction preferring microneedle patches.. In contrast, increasing the price of vaccination using a microneedle patch by $10-$20 relative to IM injection largely eliminated the increased intent to vaccinate, and most participants choosing to be vaccinated switched their preference to IM injection. This shows the strong influence of cost on participants' choice of the microneedle patch and their choice to be vaccinated.

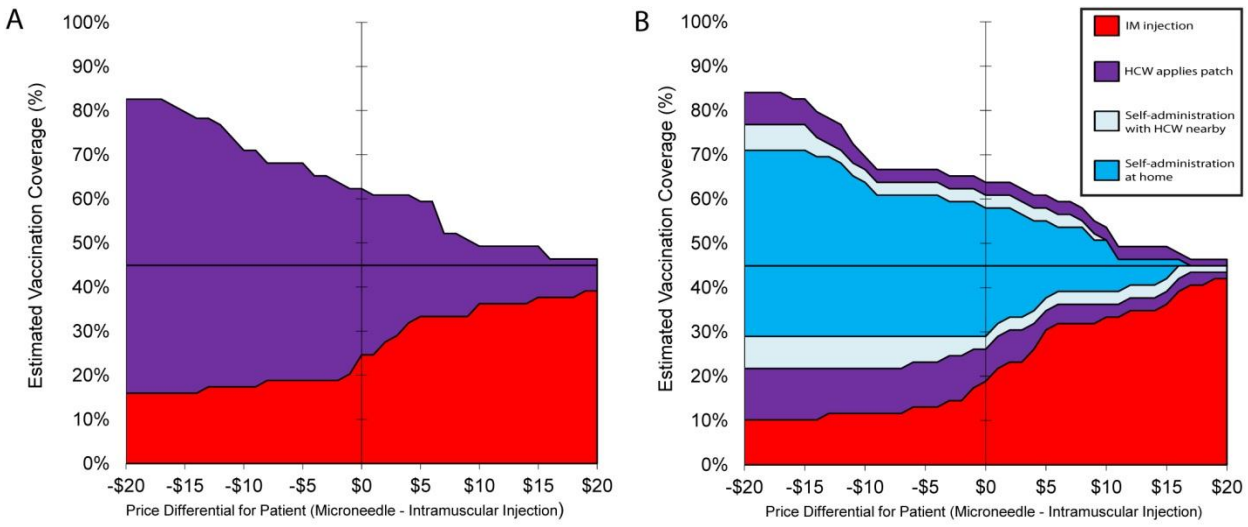

Figure 5.4 Acceptability of microneedles and self-vaccination, equal effectiveness. Participants rated their preference for patch vaccination, no vaccination, or intramuscular vaccination vs. patch price. In each graph, participants are split into those who normally intend vaccination (46%) and those who do not. (A) Acceptability of microneedles without self-vaccination versus relative patch price (B) Acceptability of microneedles with self-vaccination versus relative patch price

5.3.3 Cost-Effectiveness

We carried out an economic analysis to compare the cost-effectiveness of improving coverage with traditional vaccination methods versus improving coverage with self-administered microneedle patches using 2010 USD as the basis for comparison. We assumed that the median cost of a microneedle patch would be the same as a current injectable influenza vaccine, i.e., $10 [7]. The cost of self-administration was set equal to the median cost of administration in a pharmacy setting of $6.50, and administration by a healthcare worker was set equal to a weighted average cost of $30.

To reach the coverage levels suggested by the study, additional costs are expected because the number of vaccine recipients is increased relatively by 42%. Unsurprisingly, improving coverage with just healthcare provider administration results in a commensurate 42% increase in vaccination costs for a healthcare payer. For self-vaccination, however, our model predicts only a 4% increase in vaccination costs (CI: -15 to 24%) for the same increase in coverage. This is because self-administration is less costly than healthcare worker administration, and the cost reduction extends to those who normally intend vaccination but would switch to self-vaccination if it were available. A one-way sensitivity analysis (Figure 5.5) identified the traditional setting's administration cost and uncertainty from bootstrapping as the primary unknowns affecting uncertainty. Figure 5.6 shows two-way sensitivity analyses for changes in administration costs and uptake of self-administration.

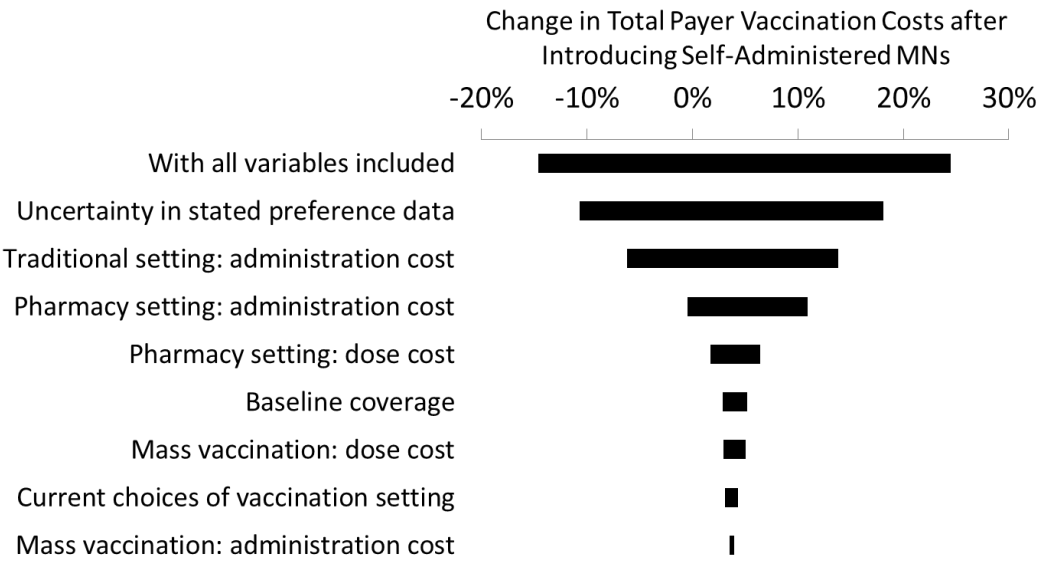

Figure 5.5 One-way sensitivity analysis for the change in total payer costs after introducing self-administered MNs. To determine which random inputs most affected the output, the model was run varying only one parameter at a time. The bars show the 95% confidence interval for each parameter's run.

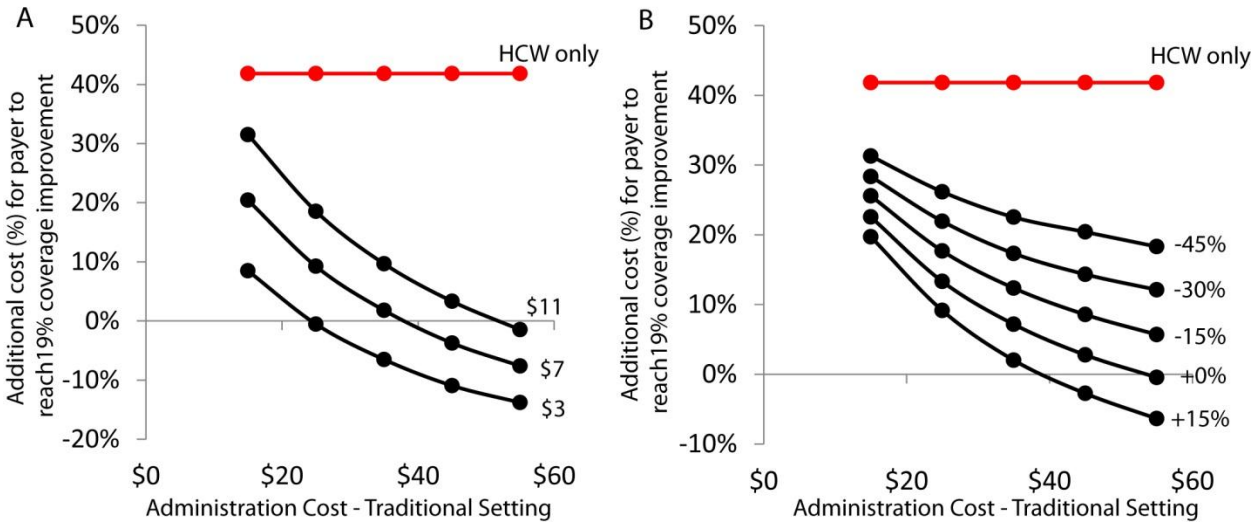

Figure 5.6 Two-way sensitivity analyses plotting additional cost for a payer to improve coverage by 19%. Each point is the median of 10,000 iterations. (A) Two-way sensitivity analysis for administration costs in the traditional setting (median: $30) and the pharmacy setting shown on multiple lines (median: $7). (B) Analysis for administration cost in the traditional setting and relative uptake of self-administration among microneedle patch users compared to the uptake measured in this study (relative to 85% uptake for new vaccinees and 72% for existing vaccinees, +15% means multiplying by 1.15x). Both figures illustrate that administration cost in the traditional setting is a major factor in the economic evaluation of self-administration. The cost of vaccination in the pharmacy setting, which was later set as the cost of self-administration, simply shifts the curve up or down. The effect of the self-adminsitration uptake rate, however, is proportional to the traditional setting administration cost. The HCW only line shows the additional cost of increasing coverage without self-administration. Values less than 0% represent an expectation of cost-savings due to self-administration.

Using the model, we also determined that a 42% increase in vaccination coverage for a typical influenza season could lead to 1.6 million fewer cases of influenza, 22,000 fewer hospitalizations, and 930 fewer deaths among adults age 18-64, who were the subject of this study. Coverage increases among children and the elderly could reduce morbidity and mortality further still.

5.3.4 Pain

We compared the pain of microneedle administration and IM injection for participants who administered their first patch correctly (Figure 5.7). The median pain scores, out of 100, were 1 for self-administration, 3 for investigator administration, and 14 for IM injection. Statistical analysis showed microneedle patch administrations were significantly less painful than IM injection (repeated measures ANOVA: $p<0.0001$; self-administration vs. IM, $p<0.01$, investigator-administration vs. IM, $p = 0.02$).

Figure 5.7 Paired comparison of pain scores. Points show paired comparisons of VAS pain scores (scale 0-100) for participants who successfully administered their first microneedle patch. The microneedle patch was significantly less painful than the IM injection for both self-administration and investigator-administration.

5.3.5 High Effectiveness Patch: Acceptability and Cost-Effectiveness

Because microneedles may offer improved immunogenicity due to vaccine targeting to dendritic cells in skin [48], we studied the impact of a hypothetical high-effectiveness patch (50% relative effectiveness improvement) on coverage and cost-effectiveness. The high-effectiveness patch improved suggested coverage levels further (Figure 5.8): 49% relative coverage improvement for the microneedle patch without self-administration (46/69 vs. 31/69) and 62% coverage improvement for a self-administered patch (50/69). We compared the cost-effectiveness of self-administration and high-effectiveness patches by calculating the reduction in average cost per case averted for different vaccination scenarios. Self-administered microneedle patches could reduce the median cost per case averted from $690 to $510. A high-effectiveness patch without self-administration could reduce that number to $540. With self-administration and high effectiveness, the median cost per case-averted could be nearly halved to $370 (see Figure 5.9).

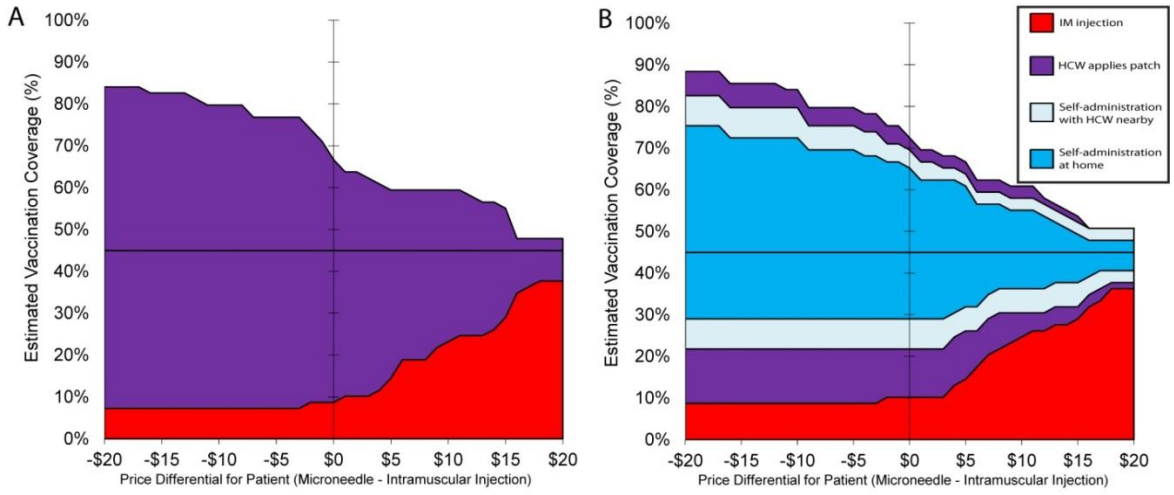

Figure 5.8. Acceptability of microneedles and self-vaccination, high-effectiveness patches. (A) Acceptability of microneedles without self-vaccination versus relative patch price. (B) Acceptability of microneedles with self-vaccination versus relative patch price.

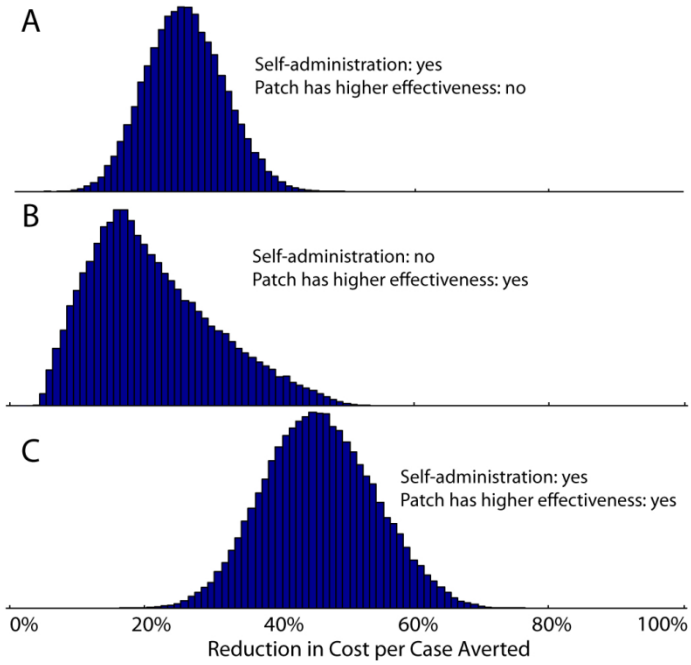

Figure 5.9 Comparison of cost-effectiveness histograms for different self-vaccination scenarios. (A) Self-adminsitration permitted, patch has same effectiveness as standard intramuscular injections. (B) Self-administration not permitted, patch has improved effectiveness. (C) Self-administration permitted, patch has improved effectiveness. Without self-administration or effectiveness improvement there is no reduction in cost per case averted in this model.

5.3.6 Factors Affecting Microneedle Patch Uptake

We measured psychosocial indicators of microneedle acceptability with constructs from the theory of reasoned action [109]. An exploratory principal components factor analysis yielded four primary factors covering 66% of the total variance among subjects (see Table 5.4). The four factors were: attitude towards microneedles, normative approval, behavioral beliefs, and outcome evaluations. Normative approval measured participants' perceived approval of doctors, family, and friends for microneedle use. Behavioral beliefs measured the perceived convenience and reliability of patches. Outcome evaluations related to physical side effects.

Each participant had a normalized score for each factor. We regressed these scores to a binary measure of whether a participant would choose a microneedle patch offered at the same price as an intramuscular injection, based on the acceptability data. The significant predictors of uptake were behavioral beliefs about usability and reliability ($p = 0.001$) and normative approval ($p = 0.02$). This indicates that acceptability of microneedles depends on convincing patients that microneedles are convenience and reliable to use and that doctors, family and friends approve of microneedle vaccination. Positive attitude towards microneedles ($p = 0.06$) and outcome evaluations ($p = 0.4$) positively correlated with uptake, but were not statistically significant, indicating that the pain reduction associated with microneedles was not important to acceptability.

The questionnaire items themselves also produced interesting results. Every participant agreed or strongly agreed that microneedle patches were easy to administer, and no participant disagreed that microneedle patches could administer flu vaccine reliably.

Table 5.4. Items Included in Behavioral Questionnaire

Factor	Mean	SD	Min	Max	Loading
Behavioral Beliefs (α = 0.845)					
1. The vaccine patch can administer flu vaccine reliably.	2.84	0.70	2	4	0.57
2. I think a vaccine patch could offer as much protection against the flu as an injected vaccine.	3.06	0.84	1	4	0.74
3. Putting a vaccine patch on my arm at home is [much less safe, less safe, as safe, safer, much safer] compared to an injection from a nurse or doctor.	2.35	0.95	0	4	0.72
4. A vaccine patch will be more convenient for me than an injection.	3.38	0.62	1	4	0.65
5. Overall, vaccine patches will help me save time compared to injections.	3.30	0.75	1	4	0.66
6. Vaccine patches are easy to use.	3.54	0.50	3	4	0.63
7. I can easily tell if I put a vaccine patch on my arm the right way.	2.86	0.99	1	4	0.60
Outcome Evaluation (α = 0.806)					
1. A vaccine patch will lead to less bleeding than an injection.	3.10	0.97	0	4	0.71
2. A vaccine patch will lead to less skin damage compared to an injection.	2.61	1.09	0	4	0.81
3. A vaccine patch will lead to less risk of injection compared to an injection.	2.65	1.04	0	4	0.78
Normative Approval (α = 0.845)					
1. My family will think it's a good idea if I choose a vaccine patch instead of an injection.	2.68	0.88	1	4	0.82
2. Important people in my life will approve if I choose a vaccine patch instead of an injection.	2.46	0.92	0	4	0.60
3. My friends will approve if I choose a vaccine patch instead of an injection.	2.81	0.77	1	4	0.79
4. My doctor will approve if I choose a vaccine patch instead of an injection.	2.51	0.76	1	4	0.66
5. Important people in my life would suggest that I get a vaccine patch instead of an injection.	2.65	1.04	0	4	0.76
Attitudes (α = 0.904)					
1. I think that vaccine patches are an improvement over injections.	3.26	0.80	0	4	0.79
2. I prefer a vaccine patch over an injection.	3.20	0.80	0	4	0.87
3. Compared to injections, vaccine patches have benefits that are important to me.	3.01	0.72	0	4	0.84
4. I like vaccine patches more than injections.	3.19	0.86	0	4	0.82
5. Putting on a vaccine patch will be less painful than getting an injection.	3.20	0.87	0	4	0.60
6. Compared to vaccine patches, injections have drawbacks that matter to me.	2.70	0.97	0	4	0.73

5.4 Discussion

This study aimed to show that self-administered microneedle patches are usable, acceptable, and potentially cost-effective. Concerning usability, almost all participants were able to self-administer microneedle patches with minimal assistance. In previous studies, microneedles have been applied to thousands of human subjects, mostly by study investigators [9]; this study showed reliable self-administration in a relatively large, diverse population. Here, many participants needed multiple attempts and instruction to push harder in order to successfully self-administer microneedles. Because of this, we think mechanical applicators may be necessary for reliable self-vaccination with microneedles. Additional work is needed to validate vaccine delivery and immunological responses after microneedle self-administration, because the staining method use here only demonstrated microneedle puncture.

For acceptability, we showed that more people would intend vaccination if a self-administered microneedle patch were available. Although self-administration was preferred over all other methods, it was not critical to increasing coverage; offering microneedle patches without self-vaccination led to most of the suggested coverage improvement. Consistent with these findings, previous studies showed that intradermal vaccination with a very small hypodermic needle was preferred [57, 205] and expected to expand vaccination coverage [204] compared to IM injection.

The quantitative acceptability analysis suggests that offering a microneedle patch alone was sufficient to convert 17% of participants to willing vaccinees, while the added convenience of self-administration had only an incremental additional effect on intent to vaccinate. However, in the behavioral analysis, the scale measuring perceived

convenience of patches was the most significant factor predicting microneedle acceptability. This suggests that self-vaccination may contribute more to coverage improvements in real vaccination situations because the convenience benefit will be tangible rather than hypothetical, as it was in our questionnaire..

Considering cost-effectiveness, we predict that a self-administered influenza-vaccine patch is an efficient and potentially cost-saving way to improve vaccination coverage.. The ability to administer more vaccine at lower cost came from reducing the cost of vaccine administration by healthcare personnel. Our study showed that participants widely preferred self-administration with microneedles over all other options, suggesting that these cost savings will come as a natural consequence of introducing microneedle patches that can be self-administered. We used the healthcare payer's perspective for a conservative estimate of cost-effectiveness. Including reduced patient time for vaccination from a societal perspective could add to the expected savings for self-vaccination.

Our evaluation assumed the microneedle patch and traditional vaccine could be sold for the same price, which is possible given the low cost of microneedle technology and the large economy of scale associated with influenza vaccine manufacturing [8]. We will need to revisit our economic analysis as microneedle manufacturing costs become clearer and as the landscape changes for vaccine presentation (e.g., prefilled syringes) [139] and vaccination settings [5].

In addition to reducing the cost of vaccination to the healthcare payer, the increased coverage resulting from introducing a microneedle patch for self-vaccination should lead to additional advantages, such as reduced patient time to be vaccinated,

increased productivity in schools and workplaces due to less illness, fewer hospitalizations, and fewer deaths. Potential negative implications include administration mistakes by patients and difficulty registering self-vaccinations into immunization information systems.

Our future research will focus first on translation. We need to improve insertion devices to approach 100% reliable insertion on the first attempt, reproduce acceptability results with larger and broader populations, conduct clinical trials on the immunogenicity and safety of self-vaccination, and scale up manufacturing. Regulatory approval will require addressing safety concerns like anaphylaxis and syncope and legal topics such as waste disposal, mailing of biologicals, over-the-counter policy, and the Vaccine Injury Compensation Program. Approval for self-administered influenza vaccines seems attainable since oral typhoid – a multi-dose, live vaccine that must be refrigerated – is also approved for self-administration [12].

The primary limitations of this study are small sample size, volunteer bias, stains that indicate microneedle insertion rather than vaccine delivery, use of stated preference for willingness-to-pay, potential bias due to experience with self-administration, and traditional biases associated with questionnaires.

CHAPTER 6

CONCLUSIONS

6.1 Fabrication of Hollow Metal Microneedles

This research presented, for the first time, a fabrication process based on sacrificial micromolding and selective electrodeposition to produce side-opened, sharp-tipped, hollow, metal microneedles. The resulting microneedles were shown to be sufficiently strong to permit reliable insertion into skin without failure and to enable injection into skin *in vitro* and *in vivo*. The micromolding approach is expected to enable low-cost, mass production of microneedles, which is an advance over prior methods that relied on direct fabrication of microneedles or more complicated molds. With additional research and development, this microneedle design could enable simple, reliable intradermal injections.

6.2 Insulin Delivery using Microneedles to Type 1 Diabetic Children and Adolescents

This is the first study of intradermal insulin delivery using microneedles in type 1 diabetic children. The study shows that microneedle administration of insulin in children resulted in less needle insertion pain and faster insulin onset and offset kinetics. The reduction in pain may increase compliance with insulin therapy, especially in needle-phobic children. The accelerated pharmacokinetics may be improve closed-loop insulin therapy, which requires rapidly responsive insulin delivery to maintain tight glycemic control.

6.3 Usability, Acceptability, and Cost-Effectiveness of Microneedle Patches for Self-Vaccination against Influenza

We conclude that microneedle patches for self-vaccination against influenza are usable, acceptable, and cost-effective. Almost all participants were able to apply microneedle patches with minimal training. Intent to vaccinate increased from 45% to 64% if a self-administered microneedle patch was offered to participants. Modeling predicted that offering self-vaccination against influenza adds only 4% to total vaccination costs even though coverage increases by 42%. Microneedles were reported to be less painful than IM injection, a hypothetical high-effectiveness patch offered for self-administration increased intent to vaccinate by a relative 45%, and perceived convenience, reliability, and normative approval were found to be the main factors affecting microneedle patch uptake by participants. This first study on self-vaccination for coverage and cost-effectiveness improvements should motivate further research and translational activities in this area.

CHAPTER 7

RECOMMENDATIONS

7.1 Recommendations for Hollow Microneedle Design

A key remaining challenge in hollow microneedle design is to determine if side-opened microneedles reduce the pressure of delivery compared to microneedles with axial lumens. An adequately controlled experiment has not been performed. This is important because reduced pressure will reduce the demands on the pumping mechanism [69] and because reduce pressure is expected to reduce pain associated with infusion [67]. Another challenge is designing microneedles to integrate with discrete, wearable insulin pumps and stay in a patient's skin for multiple hours' use. This is important because the full potential of microneedles will be realized by a patch-like system for infusion into skin, as opposed to simply mounting microneedles onto conventional syringes, as is currently done.

7.2 Recommendations for Insulin Delivery in Human Subjects

Four recommendations came from the insulin delivery study in type 1 diabetic children. First, because microneedles were more painful during infusion compared to subcutaneous catheters, research should focus on reducing the pain of intradermal injections. This can be accomplished by adding the spreading enzyme hyaluronidase [67], reducing the flow rate, using multiple injections, or using an optimzied programmed flow rate pattern to infuse liquids into skin at minimal pressure. Second, long-term studies should be conducted to examine the acceptability of microneedles and their effect on injection compliance in adults and children. Improving compliance is a major reason for

investigating microneedels for insulin delivery, and compliance can be quantitatively assessed by measuring HbA1C levels after experimental use of a device [179]. Third, research should focus on accelerating the pharmacokinetics of insulin delivery even further by using insulin analogs, applying local heating, or distributing the bleb across multiple injection sites to facilitate protein uptake. Finally, studies in animals and humans should test whether microneedle-based insulin delivery can truly enable closed-loop insulin delivery. It is hypothesized that the rapid onset and offset of insulin delivery will facilitate automatic control of plasma glucose levels, but this has yet to be tested.

7.3 Recommendations for Self-Vaccination

Future research on self-vaccination should focus on solving problems on the path to regulatory approval and then expand self-administration to other vaccination programs. The problems on the path to approval include improving the first-attempt reliability of self-administration by using applicators, confirming successful drug or antigen delivery after self-administration, validating the acceptability results in a broader population, validating potential compliance with self-vaccination, addressing legal issues like waste disposal and prescription policies, and addressing safety issues like anaphylaxis and syncope.

Expanding self-vaccination beyond influenza vaccines could include evaluating self-administration of other voluntary vaccines, evaluating administration by minimally-trained vaccinators in the developing world, and developing novel prime-boost regimens made possible by self-vaccination.

7.4 General Recommendations for Microneedles

This section considers recommendations for microneedle research beyond the scope of this particular dissertation. Future microneedle work will have three foci: developing microneedles as marketable products for well-studied clinical indications (e.g. insulin delivery), applying microneedles to known drug delivery problems, and developing microneedles for new applications.

Developing microneedles as marketable products encompasses the obvious but difficult work of finalizing a product design and obtaining regulatory approval for a disruptive technology.

For applying microneedles to known problems, examples include immunization of the young and the old, studies of the efficacy of heterologous prime-boost immunizations using two different sites of antigen delivery[212, 213], and comparative effectiveness studies comparing the efficacy and feasibility of multiple vaccination methods.

Developing new applications for any device is a challenge. New applications for microneedles should draw from the core advantages of microneedles over other drug delivery methods: targeting to specific tissues; improved patient compliance; administration by patients or by minimally trained personnel; delivery of larger doses than the transdermal route; delivery of more consistent doses than the mucosal route; and, ability to use complex delivery profiles based on formulation, programmed pressure sources, or user input [214]. One application may be microneedles for blinding trachoma if the disease is not eliminated by 2020 as expected [215]. Microneedles would be useful

for targeting azithromycin to the conjunctiva to reduce dose requirements and community antibiotic resistance, while also enabling administration by minimally trained personnel.

Microneedles may be useful for treating and preventing other infections with antimicrobials, as sustained transcutaneous delivery may keep antimicrobial concentrations above minimum inhibitory concentrations [216] with minimal patient effort required; the key challenge may be storing and delivering sufficient doses of antimicrobials.

Diagnostic applications of microneedles seem promising and may even be useful in epidemiological research by permitting quick identification of target individuals [217]. Microneedles will likely find non-medical (commercial and military [218]) applications for on-demand or automated drug delivery. Finally, I expect the skin microbiome will garner some attention from microneedle researchers for the possibility of preventing and treating certain illnesses [219].

APPENDIX A

DETAILED METHODS

A.1 Molding Process for Hollow Metal Microneedles

A.1.1 Quantifying Percent of Dye Delivered Intradermally Using ImageJ

Fluorescent images of histological cross-sections were opened in ImageJ, and split into red, green, and blue channels (Image → Color → Split Channels). Only the red channel was kept for analysis. The free-hand selection tool was used to select background areas, dermis areas, and non-dermal-tissue areas identified in light microscopy images. Figure A.1 shows an example background selection on a red channel image.

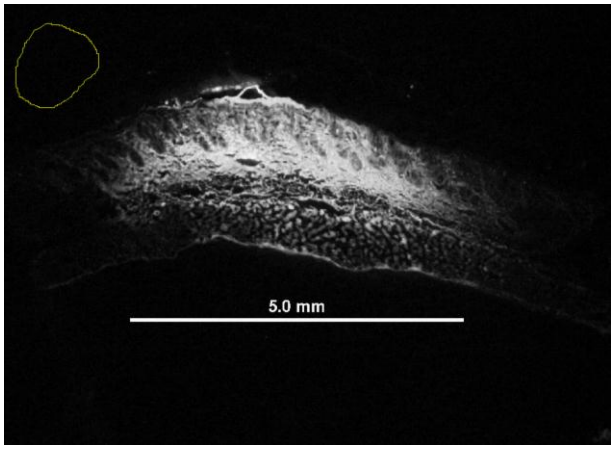

Figure A.1 Selecting a background region on a red channel image

For each region selected, after clicking on (Analyze → Measure), ImageJ provides an area and an average intensity. The area and average intensity can be used to quantify the percent of dye localized intradermally using the following formula.

$$\%_{ID} = \frac{A_{ID}(I_{ID} - I_{background})}{A_{ID}(I_{ID} - I_{background}) + A_{nonID}(I_{nonID} - I_{background})} \quad \text{Eq. A.1}$$

In this equation, A represents the area of a particular region, I represents the mean red intensity of a particular region, ID is intradermal, and nonID is non-intradermal tissue.

A.2 Insulin Delivery Using Microneedles to Type 1 Diabetic Children and Adolescents

A.2.1 Regressing Insulin Concentrations to a Compartment Model Equation

Insulin concentration over time for each participant was fit to a one-compartment model assuming a constant absorption rate from an injection site and a constant elimination rate. The model is derived from the following system of differential equations [220]:

$$\frac{dI}{dt} = -k_a \cdot I \quad \text{Eq. A.2}$$

$$\frac{dC}{dt} = k_a \cdot I - k_e \cdot C \quad \text{Eq. A.3}$$

$$I_{t=0} = I_0 \quad \text{Eq. A.4}$$

$$C_{t=0} = 0 \quad \text{Eq. A.5}$$

Here, I is the concentration of insulin at the injection site (μU/ml), I_0 is the dose of insulin delivered (μU), k_a is the absorption rate (min^{-1}), k_e is the elimination rate (min^{-1}), t is time (min), and C is the concentration of insulin in the systemic volume (μU/mL). The

solution for C(t) is given below:

$$C(t) = \frac{A \cdot k_a}{k_e - k_a}(e^{-k_a \cdot t} - e^{-k_e \cdot t}) \qquad \text{Eq. A.6}$$

where, and A is a scaling parameter (μU/mL) equal to I_0 divided by the systemic volume.

Insulin concentrations were fit using proc nlin in the SAS software package. Data for each condition for each participant was put in manually into a variable called insulinC. Example SAS code for data entry and regression are shown below:

```
data insulinC;
input time conc;
datalines;
0       0
15      48.5
30      71.8
45      68.2
60      55.8
75      46.1
90      37.8
105     33.5
120     26.1
150     20.8
180     15.3
210     11.7
240     9.5
;
run;

proc nlin data=insulinC;
  parameters  A=200 ka=.01 ke=.03;
  model conc   = A*ka/(ke-ka)*(exp(-ka*time)-exp(-ke*time));
run;
```

Occasionally the model would not converge in situations where k_a and k_e were nearly equivalent. In these cases, A was removed as a regression parameter and manually iterated until the squared error of the model was minimized. The final output for the

regression was values for A, k_a, and k_e for each experimental condition for each participant.

A.2.2 Calculating Onset and Offset Times

In Chapter 4, insulin onset time was defined as the time to reach the peak insulin concentration, and insulin offset time was defined as the time to fall back to half the peak concentration. These values were calculated using the A, k_a, and k_e values from regression analysis. The time to peak concentration can be setting the derivative of the concentration vs. time equation equal to zero.

$$\frac{dC}{dt} = \frac{A \cdot k_a}{k_e - k_a}(k_e \cdot e^{-ket} - k_a \cdot e^{-k_a \cdot t}) = 0 \qquad \text{Eq. A.7}$$

$$k_e \cdot e^{-ket} - k_a \cdot e^{-k_a \cdot t} = 0 \qquad \text{Eq. A.8}$$

$$k_e \cdot e^{-ket} = k_a \cdot e^{-k_a \cdot t} \qquad \text{Eq. A.9}$$

$$\ln(k_e / k_a) = (k_e - k_a) \cdot t \qquad \text{Eq. A.10}$$

$$t_{C(t)=\max} = t_{\max} = \frac{\ln(k_e / k_a)}{k_e - k_a} \qquad \text{Eq. A.11}$$

The time to peak concentration depends only on the rate constants in this simplified model. There is no dependence on the size of the dose delivered. For cases where k_a and k_e are close (and represented by a single rate constant: k):

$$\lim_{k_a \to k_e} t_{\max} = \frac{1}{k} \qquad \text{Eq. A.12}$$

Plugging t_{max}, the time to peak concentration, into Eq. A.6 provides the peak concentration, C_{max}:

$$C_{\max} = C(t_{\max}) \quad \text{Eq. A.13}$$

$$C_{\max} = \frac{A \cdot k_a}{k_e - k_a}\left(e^{-k_a \cdot \frac{\ln(k_e/k_a)}{k_e - k_a}} - e^{-k_e \cdot \frac{\ln(k_e/k_a)}{k_e - k_a}}\right) \quad \text{Eq. A.14}$$

$$C_{\max} = \frac{A \cdot k_a}{k_e - k_a}\left[\left(\frac{k_e}{k_a}\right)^{\frac{-k_a}{k_e - k_a}} - \left(\frac{k_e}{k_a}\right)^{\frac{-k_e}{k_e - k_a}}\right] \quad \text{Eq. A.15}$$

$$C_{\max} = \frac{A \cdot k_a}{k_e - k_a}\left[\left(\frac{k_e}{k_a}\right)^{\frac{k_e}{k_a - k_e}}\left(\frac{k_e}{k_a}\right)^{\frac{(k_a - k_e)}{k_a - k_e}} - \left(\frac{k_e}{k_a}\right)^{\frac{k_e}{k_a - k_e}}\right] \quad \text{Eq. A.16}$$

$$C_{\max} = \frac{A \cdot k_a}{k_e - k_a}\left[\left(\frac{k_e}{k_a}\right)^{\frac{k_e}{k_e - k_a}}\right] \cdot \left(\frac{k_e}{k_a} - 1\right) \quad \text{Eq. A.17}$$

$$C_{\max} = \frac{A \cdot k_a}{k_e - k_a}\left[\left(\frac{k_e}{k_a}\right)^{\frac{k_e}{k_e - k_a}}\right] \cdot \left(\frac{k_e - k_a}{k_a}\right) \quad \text{Eq. A.18}$$

$$C_{\max} = A \cdot \left(\frac{k_e}{k_a}\right)^{\frac{k_e}{k_e - k_a}} \quad \text{Eq. A.19}$$

The insulin offset time was solved for numerically using Microsoft Excel's solver function. The value for t in Eq. A.6 was iterated until the concentration from that equation was $0.5*C_{max}$. Because $0.5*C_{max}$ and Eq. A.6 have the same linear relationship with A, A can be divided from both sides of the equation. Because A does not remain in the equation, the offset time in this model also does not depend on the size of the dose delivered.

A.2.3 Calculating Area Under the Insulin Curve

The area under the insulin curve (AUC) is a measurement for comparing the bioavailability of two insulin delivery methods. It is the integral of insulin concentration vs. time. The integral can be approximated using the trapezoid rule, or calculated exactly from the compartment model equation A.6. Both integrals are shown below.

$$AUC_{approx} = \sum_{n=1}^{n} [\frac{C_n + C_{n-1}}{2} \cdot (t_n - t_{n-1})] \quad \text{Eq. A.20}$$

$$AUC_{exact} = \int_0^{\infty} C(t)dt \quad \text{Eq. A.21}$$

$$AUC_{exact} = \frac{Ak_a}{k_e - k_a} \int_0^{\infty} (e^{-k_a \cdot t} - e^{-k_e \cdot t})dt \quad \text{Eq. A.22}$$

$$AUC_{exact} = \frac{Ak_a}{k_e - k_a} \cdot (\frac{1}{k_a} - \frac{1}{k_e}) \quad \text{Eq. A.23}$$

$$AUC_{exact} = \frac{Ak_a}{k_e - k_a} \cdot (\frac{k_e - k_a}{k_a \cdot k_e}) \quad \text{Eq. A.24}$$

$$AUC_{exact} = \frac{A}{k_e} \quad \text{Eq. A.25}$$

The exact integral does not depend on the absorption rate. Therefore, delivery methods with different absorption rates such as intradermal and subcutaneous delivery should have similar bioavailabilities according to this model.

A.3 Usability, Acceptability, and Cost-Effectiveness of Microneedle Patches for Self-Vaccination against Influenza

A.3.1 Patch Fabrication

Microneedle patches were made from four parts: a stainless steel microneedle array, an adhesive foam backing, a polyacetal packaging piece, and a paper/silicone liner. Bending needles, cutting parts with a CO2 laser, and cleaning parts took place in an area of the lab portioned off by plastic drapes. The remainder of the processes took place in a clean laminar flow hood with lab personnel wearing clean disposable gowns, sleeve covers, and gloves.

A.3.1.1 Bending the Arrays Out-of-Plane

Stainless steel microneedle arrays were purchased from a contract manufacturer and bent 90° out-of-plane. Four joined arrays were loaded into a laser-cut acrylic mold with holes positioned below the needles. The arrays were taped into place using a medical grade cloth tape. A 25 gauge, 5/8" long sterile needle attached to a 3 mL syringe was used to press each needle out-of-plane. The partially bent arrays were placed under a microscope, and a number-11 scalpel blade was used to bend rows of needles to 90°. Groups of four joined arrays were then cut into single arrays using stainless steel scissors. Microneedle arrays were cleaned three times in isopropyl alcohol prior to assembly.

A.3.1.2 Cutting out the Backing

30 mm adhesive foam backings were made from a medical grade adhesive foam material (TM9942, MacTac, Stow, OH). The adhesive foam backings were cut out using an arbor press (2402A11, McMaster Carr, Atlanta) modified to have a hollow punch tool (66004, Mayhew Tools, Turner Falls, MA) placed on the piston.

A.3.1.3 Cutting Out the Packaging Material

Polyacetal packing pieces were made using a CO2 laser to cut 3/32" polyacetal sheeting. The shape is shown in Figure A.2 in black. The shape was designed to be cut out using only straight lines and circles in order to accommodate a paper/silicone liner, which could only be cut using scissors and the hollow punch tool on the arbor press. The shape is defined as a 45x30 mm rectangle with multiple circular cuts. Two 30 mm semicircular cuts defined a rounded shape for the packaging. A partial 24 mm circular cut defined a place for user to pick up the patch, and a 16 mm hole provided a window where the needles sit surrounded by polyacetal packaging. The polyacetal pieces were cleaned three times in isopropyl alcohol before assembly.

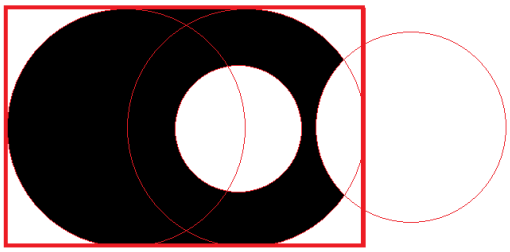

Figure A.2 Diagram showing packaging and liner shape for microneedle patch and the circles used to define the shape using a hollow punch tool

A.3.1.4 Cutting out the Liner

A liner material is necessary because the adhesive foam backing and the packing material adhere too strongly to each other. A liner material is cut out from the liner material from the adhesive foam backing (TM9942, MacTac, Stow, OH) with a thin double-stick adhesive (1522, 3M, Minneapolis) applied beforehand.

A.3.1.4 Assembly and Sterilization

Each liner is adhered to a polyacetal packaging piece using the double-stick adhesive on the liner. An adhesive foam backing is set into place on the liner side of the polyacetal packing. Then, a microneedle array is set into the 16 mm window of the packaging piece and pressed into place with tweezers. Fig 5.1A shows an example complete device.

Completed devices were double-bagged in manually sealed sterilization pouches (Thermo Fisher Scientific, Waltham, MA). Bagged devices were placed into cardboard shipping boxes with packaging paper added to space devices evenly for ethylene oxide sterilization. Boxes were sent to Steris Isomedix (Minneapolis, MN) for two ethylene oxide sterilization cycles.

A.3.2 Venue Recruiting: List of Locations and Materials Used

Participants were recruited from publically accessible venues. A list of venues for each month was prepared beforehand containing random venues (available at any time during a month) and non-random venues (special events only available on certain dates). The list of venues included sites used by Emory University's Hope Clinic for other studies, sites recommended by the Hope Clinic to attain a good geographic distribution of sites within 5 miles of the Georgia Tech campus, non-random sporting events, and non-random events collected from Atlanta festival websites and other sources. Specific locations could have more than one available timeframe, but each location would only be used once per month. An example list of venues is shown below in Table A.1.

Table A.1 Venue Pool used in October 2011

MARTA - Buckhead	MARTA - Oakland City
MARTA - Lindbergh	MARTA - West Lake
MARTA - Arts Center	MARTA - Ashby
MARTA - Midtown	MARTA - Vine City
Sweetwater Brewery	Centennial Olympic Park Area
Clifton and North Decatur Starbucks	Pittman Park Rec Center
Street Food Thursdays - 10th and Peachtree	Windsor Super Market / Dunbar Center
1010 Midtown building area	Sweet Auburn Curb Market
Piedmont Park	Ria's Bluebird / Mibarrio
MARTA - Lenox	Thumbs Up Diner
Flying Biscuit / Caribou	Nick's Greek Food to Go downtown
Ponce + Myrtle (Mary Mac's Tea Room)	MARTA - North Avenue
Linden and Peachtree	MARTA - Civic Center
Ansley Park Shopping Center	MARTA - Peachtree Center
Peachtree between Collier and Montclair	MARTA - Five Points
Fellini's Buckhead near Episcopal Cathedral	MARTA - East Lake
Publix or CVS at Lindbergh and Cheshire Bridge	MARTA - Edgewood/Candler Park
Coffee Shops near Peachtree and 17th	MARTA - Inman/Reynoldstown
Virginia and Highland Intersection	MARTA - King Memorial
Highland and University (Yoforia, etc.)	MARTA - Georgia State
Sage Hill Shopping Center	Grant Park / Zoo
High Museum / Colony Square lunch time	Little 5 Points
Atlantic Station	Candler Park Neighborhood
11th and Howell Mill	East Atlanta, Main Intersection
Taqueria del Sol on Howell Mill	Edgewood Shopping Center
10th and Atlantic	MARTA - Bankhead
Paces Ferry Plaza Shopping Center	Inman Park
Howell Mill + Collier	Center for Black Women's Wellness
Nuevo Laredo Cantina	Purple Door Salon
Octane Coffee Bar/Five Seasons	Woodruff Park
Bobby Jones Golf Course/Tennis Center	Dannemann's Restaurant
Firehouse subs / Sublime Donuts	Sugar Hair Studio
Carvers Country Kitchen	Little 5 Points Halloween Festival
Redbrick (ABC) brewing company	Atlanta Pride Festival
MARTA - Garnett	Taste of Atlanta
MARTA - West End	Philips Arena Show or Basketball Game
Georgia Tech Football Game	Fox Theater
Falcons Game	CW Center Stage

A calendar was prepared each month according to previously published procedures [221]. We compiled potential venue timeframes into a .csv file, and created a Visual C# program to generate a calendar according to these procedures. In the .csv, the first column was the venue name, the second column was the date (an abbreviation such as 'tu' for Tuesday or a decimal such as 10.08 for October 8), the third and fourth columns had beginning and ending times for recruiting at that venue listed in military time format (e.g. 1500 for 3:00 PM). The program provided a primary and two alternate venues for each day of the month.

We approached people at recruiting sites with a standard message:

> "Hello, I'm recruiting for a study at Georgia Tech. Can I talk with you for a minute? We're studying a new type of skin patch and comparing it to an intramuscular injection. This is a device comparison study, so there are no drugs involved. You would come to Georgia Tech for an hour, and if you're not a Georgia Tech student or employee, you will be given $35 for your participation. The study involves putting the patch on yourself, have us put it on you, and have us give you an injection. We will apply a dye to test whether the patch was applied correctly. You would also answer some questions about pain you felt, your preferences for different procedures, your thoughts on how to improve the skin patch, your experience with flu vaccines, and your personal characteristics, such as your age and gender. If you are interested, I would like to try to schedule you for an appointment. We can only schedule you for an appointment if you meet these requirements [Investigator shows exclusion criteria]. I would like to collect your first name, last initial, phone number, and e-mail address as well."

If a person was interested, we collected their name and phone number and provided them with a card, an appointment time, and a map to the study location at Georgia Tech. An image of the card is shown below in Figure A.3.

Figure A.3 Business card used during venue based recruiting

A.3.3 Usability Analysis

Participant skin sites were stained after microneedle insertion in order to quantify the usability of microneedle patches. Example stains are shown in Figure 5.1C and Figure 5.1D. Some factors affecting quantification included blood spots or other indeterminate spots and unquantifiable images. Some images were unquantifiable due to excess staining with fluorescein or insufficient lighting to observe the fluorescent spots (e.g., when the batteries to the lighting did not provide sufficient power). The formula used for quantifying usability was:

$$\%_{inserted} = \frac{N_{counted}}{50 - N_{uncountable}} \qquad \text{Eq. A.25}$$

An example image with indeterminate blood spots considered uncountable is shown in Figure A.4. An example unquantifiable image is shown in Figure A.5.

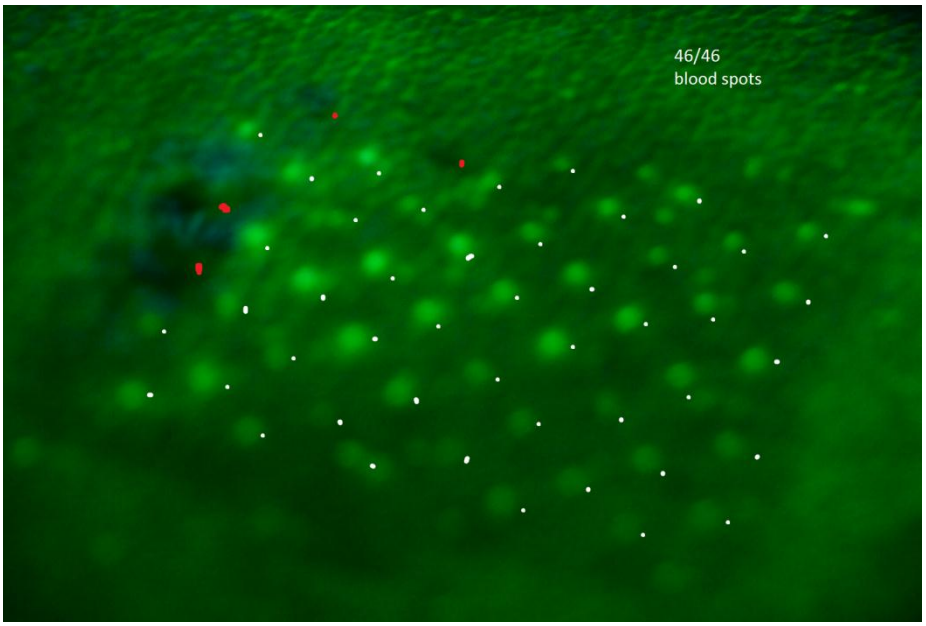

Figure A.4 Example stain with uncountable, indeterminate blood spots

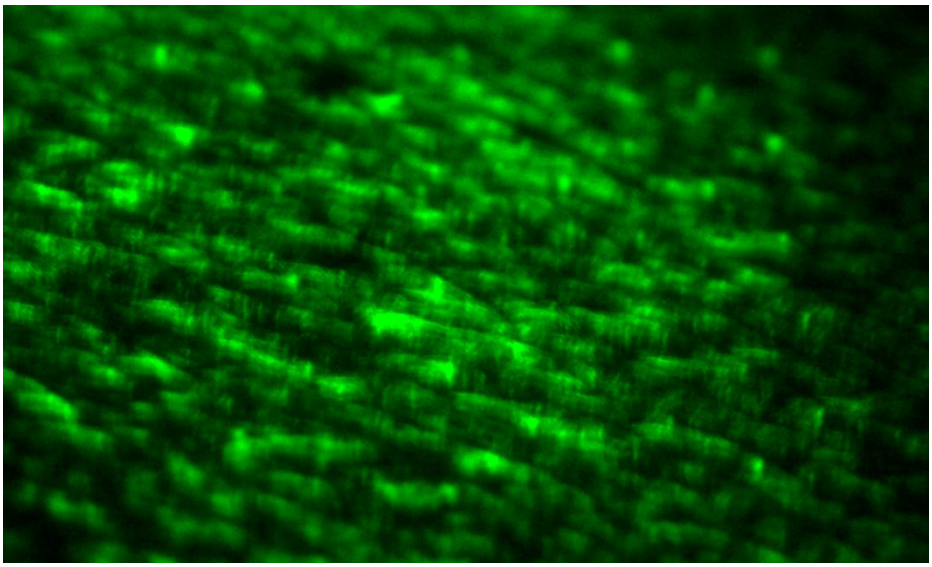

Figure A.5 Example unquantifiable fluorescein stain

Some original images of fluorescein stains were dark and difficult to quantify without modifying the color balance. The color balance on these images was linearly modified over the whole image in order to visualize the insertion sites. An example of this modification is shown in Figure A.6 and Figure A.7.

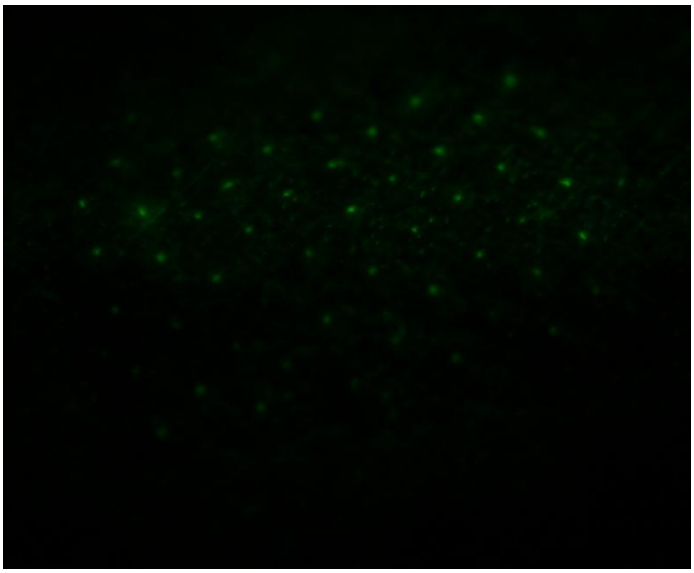

Figure A.6 Fluorescein stain before color balance adjustment

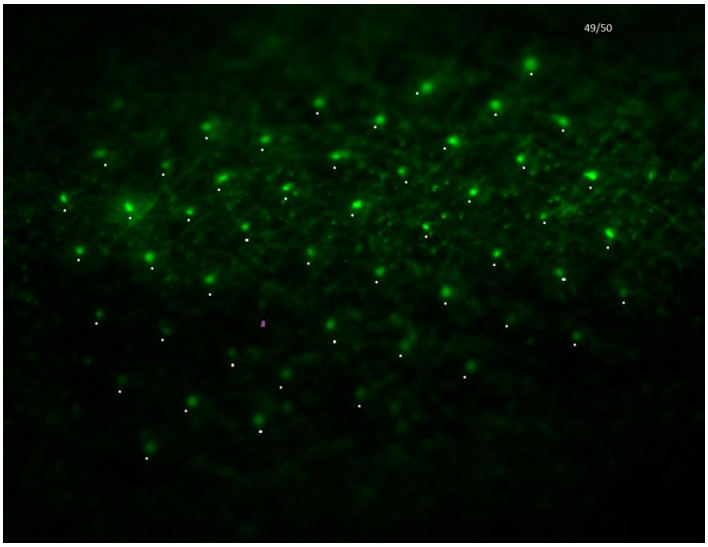

Figure A.7 Fluorescein stain after color balance adjustment

A.3.4 List of Questionnaire Materials

Participants viewed movies and answered questionnaire items using an adaptive questionnaire program developed in Visual C# (Microsoft, Redmond, WA). Movies were created using Corel Visual Studio Pro (Ottawa, ON).

A.3.4.1 Introductory Movie

Before beginning experiments, participants viewed an introductory movie with the following script:

> "Welcome to Georgia Tech's device comparison study. We appreciate you taking your time to participate. Here is an overview of what to expect today: First, for the device comparison study, you will be comparing two medical devices: (1) a standard needle and syringe, and (2) a new type of skin patch. You will experience three different ways that these devices can be used. No drugs or vaccines will be used in this study, however. We are only studying your experience with the devices. The three different administration methods we're studying are: (1) we give you an injection, (2) we put the skin patch to you, and (3) you put the skin patch to yourself. You will be given three skin patches to apply to yourself. You will experience these methods in a random order. After each method you will be asked to rate your pain on a sliding scale. After the skin patch insertions, we will be applying a dye to your skin to determine whether the patch was successfully administered. This part of the study should not take more than 20 minutes. After the device comparison study, you will be asked to complete a short questionnaire related to your experience and what you think about these devices being used for flu vaccines. The survey should not take more than 30 minutes to complete. As we move on, please answer each question as honestly as possible. You may choose not to participate, and you may skip any question you do not wish to answer. However, please try to answer every question. Thank you again for your participation. Let's begin."

A.3.4.2 Pain Assessment

Volunteers rated the pain associated with the procedures in the study using a sliding bar ranging from "No pain" to "Worst Pain." Procedure Labels: [when we gave you an injection, when we put the vaccine patch on you, when you put on the vaccine patch].

A.3.4.3 Definition Movies

Movies were presented to participants to define terms in the questionnaire.

- Flu/Influenza: "The flu is a disease that affects people primarily in Fall and Winter, and a different form of this disease comes back every year. A vaccine that helps prevent the flu is available every year."
- Injection: "An injection is the delivery of fluid using a needle and syringe. An injection is the most common way to deliver the seasonal flu vaccine."
- Vaccine Patch: "The vaccine patch is a device that can contain vaccine that dissolves in the skin. Vaccine patches are being studied as a method for delivering flu vaccines."
- Healthcare Worker: "A healthcare worker is a nurse, a doctor, or a pharmacist who can deliver a vaccine to you. All current flu vaccines are given by healthcare workers."
- Self-Administration: "People may be able to deliver vaccines to themselves in the future using technology like the vaccine patch. You may be able to purchase a vaccine at a pharmacy or grocery store or from an online store and deliver it to yourself at home. Another option is self-administration of a vaccine with a healthcare worker nearby who will make sure you performed the vaccination correctly. This could be done at pharmacies, workplaces, community centers, or any other place where flu shots are typically given. Self-administration is expected to be more convenient that healthcare worker administration because vaccines will be available in more locations and there will be less waiting in lines. Also, self-administration at

home could be done at any time during the day rather than only when a healthcare worker is available."

A.3.4.4 Demographic Questions

- What is your gender? [Male, Female, Transgender]
- What is the highest level of education you have completed? [Have not completed kindergarten, K-8 grade, 9-11 grade, High School Graduate / GED, Technical/Vocational or Associate's Degree, Bachelor's Degree, Master's Degree, Doctorate Degree]
- How would you describe your race/ethnicity? [Asian/Asian American/Pacific Islander, Hispanic/Latino/Chicano, African American/Black, Caucasian/White, Native American/American Indian/Alaskan Native, Multiracial]
- What is your annual household income (combined income of all members of your household)? [Less than $20,000, $20,001-40,000, $40,001 - $60,000, $60,001 - $80,000, $80,001 - $100,000, more than $100,000]
- What is your age in years? [open ended]

A.3.4.5 Questions on Flu Vaccine Experience (Adaptive)

- Have you ever had a case of the flu? [Yes, No, Don't Know]
- Have you received a flu vaccine in the past 3 years? [Yes, No, Don't Know]
    - If yes: Approximately what price did you pay for your flu vaccine? [open ended]

- Otherwise: Approximately what price would you have to pay for a flu vaccine? [open ended]

- Do you intend to get a flu vaccine in the next 12 months? [Yes, No]

A.3.4.6 Movie Explaining Stated Willingness-to-Pay Section

Before the next section of the questionnaire, the stated willingness-to-pay-section, participants were shown a movie explaining how to respond to questions in that section. The script of the movie was:

> "In the next section, you will be asked about your preference for 3 different vaccination methods. On the screen you will be told that you can get a flu vaccine by injection for a certain price, choose not to get a flu vaccine at all, or choose the option below. The starting price for each vaccination method is random, and it changes based on your answers. Please click a button indicating whether you would or would not accept the displayed option. In total, there are 12 questions in this section."

A.3.4.7 Stated Willingness-to-Pay Questions (Adaptive)

Volunteers express their willingness-to-pay for the following scenarios relative to a control of intramuscular injection or no vaccination: use a vaccine patch at home, use a vaccine patch with a healthcare worker nearby, and have a healthcare worker apply a patch to you. For each scenario, there were four questions based on a binary search algorithm to find the participants maximum willingness-to-pay value. The range of prices search went from (base cost - $20) and (base cost + $20), where "base cost" is the minimum of $25 or the volunteer's reported price they pay for their flu shot. The starting guess was a random number between (base cost - $12) and (base cost + $12), and all

prices shown to the participant were rounded to the nearest dollar. An example frame from this section of the questionnaire is shown in Figure A.6.

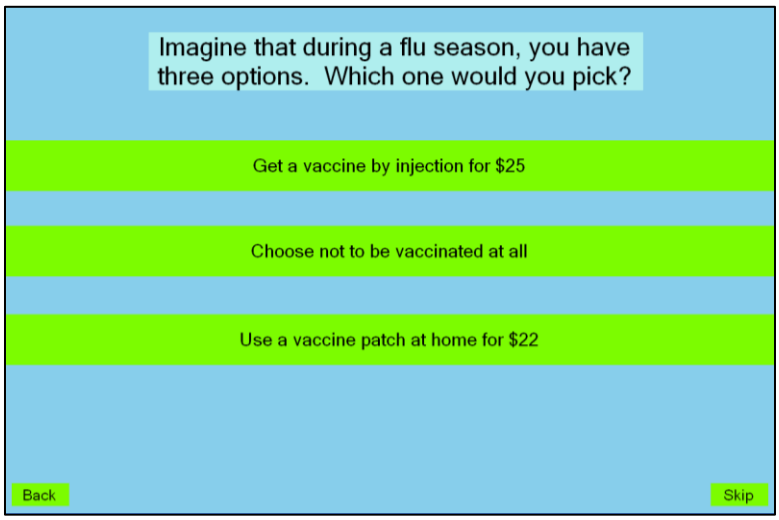

Figure A.6 Example Frame from Willingness-to-Pay Questionnaire

If a participant accepted a patch option at every price shown, they were asked to list their open-ended willingness to pay as shown in Figure A.7.

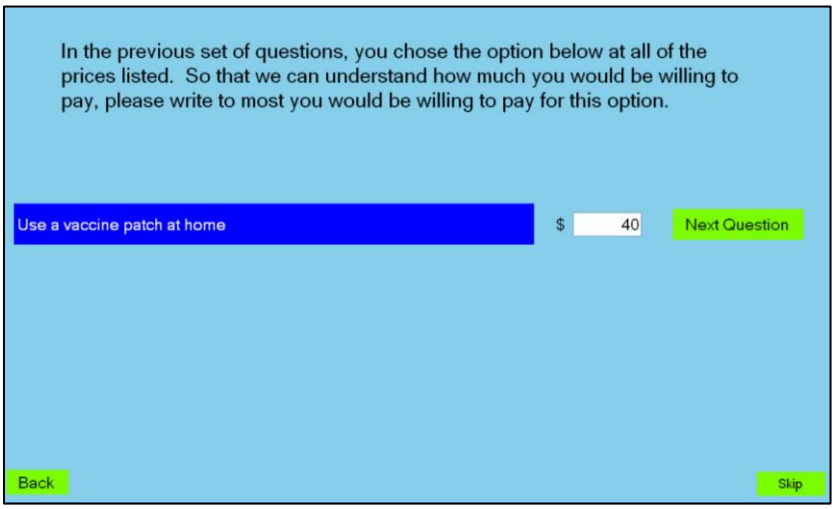

Figure A.7 Example Frame for Open-Ended Willingness to Pay

A.3.4.8 Stated Willingness-to-Pay for High Protection Patches

Participants repeated the stated willingness-to-pay section to evaluate a hypothetical "high-protection" (high-effectiveness) vaccine patch in different use scenarios. The difference in protection level was described in a movie with the following script:

> "Receiving a flu vaccine does not guarantee protection against the flu. A few vaccine recipients will still get the flu. Newer vaccines may offer better protection against the flu. For new, high protection vaccines: assume you would have a smaller chance of getting the flu, a 50% smaller chance. Please answer 12 more preference questions, this time, all of the options will offer high protection."

A.3.4.9 Preferred Method

After the stated willingness-to-pay section, participants chose a preferred method of vaccination out of the following options: use a vaccine patch at home, use a vaccine patch with a healthcare worker nearby, have a healthcare worker apply a patch to you, have a healthcare worker give you an injection. For five participants for whom this information was unavailable, preferred method was imputed by taking the method with the maximum willingness-to-pay.

A.3.4.10 Behavioral Questions Based on the Theory of Reasoned Action

Participants responded to questions based on the theory of reasoned action shown in Table A.2, with all items measured on a 5-point Likert scale.

Table A.2 Questions based on the Theory of Reasoned Action

Item
Important people in my life will approve if I choose a vaccine patch instead of an injection.
My family will think it's a good idea if I choose a vaccine patch instead of an injection.
My friends will approve if I choose a vaccine patch instead of an injection.
Important people in my life would suggest that I get a vaccine patch instead of an injection.
My doctor will approve if I choose a vaccine patch instead of an injection.
I think that vaccine patches are an improvement over injections.
Compared to injections, vaccine patches have benefits that are important to me.
I prefer a vaccine patch over an injection.
Compared to vaccine patches, injections have drawbacks that matter to me.
I like vaccine patches more than injections.
Putting on a vaccine patch will be less painful than getting an injection.
A vaccine patch will lead to less bleeding than an injection.
A vaccine patch will lead to less skin damage compared to an injection.
A vaccine patch will lead to less risk of infection compared to an injection.
I think a vaccine patch could offer as much protection against the flu as an injected vaccine.
Putting a vaccine patch on my arm at home is [less safe, as safe, safer] compared to an injection from a nurse or doctor.
A vaccine patch will be more convenient for me than an injection.
Overall, vaccine patches will help me save time compared to injections.
It will help me if the flu vaccine is available in more locations.
Vaccine patches are easy to use.
I can easily tell if I put a vaccine patch onto my arm the right way.
The vaccine patch can administer flu vaccine reliably.

A.3.5 Acceptance Analysis

A.3.5.1 Excluding Self-Administration

We used the stated willingness-to-pay data to define a maximum price for each participant where they would prefer a microneedle patch administered by a healthcare worker over an intramuscular injection or no vaccination. For participants who did not accept the microneedle patch at any price, we arbitrarily defined their maximum acceptable price for at $-1000.

Participants who normally intend vaccination and had a maximum acceptable price greater than or equal to their estimated intramuscular injection price were assumed to choose microneedle patches if both options were available at the same price. The remainder of participants who normally intend vaccination were assumed to choose intramuscular injection.

In the same way, participants who do not normally intend vaccination were binned into those who would accept a microneedle patch and those who would remain unvaccinated if a microneedle patch was available at the same price as an intramuscular injection. See Figure 5.3A for a pie chart showing how participants fell into each category.

This analysis could be repeated for scenarios where the microneedle patch would be more or less expensive than an intramuscular injection, as shown in Chapter 5.

A.3.5.1 Including Self-Administration

To analyze acceptance if self-administration was available, we relied on each participant's chosen, preferred method. The approach was similar to the approach section A.3.5.1, but the maximum price for each participant was based on their preferred method. The pie chart in Figure 5.3B includes self-administration options as separate categories and illustrates that several people who accepted microneedle patches without self-administration would prefer a self-administered patches if they were available.

A.3.6 Economic Analysis

A quantitative economic analysis was conducted using the acceptability data. The purpose of this economic analysis was the compare hypothetical scenarios with the introduction of microneedle patch or a self-administered microneedle patch to the current influenza vaccination program in the United States. Several outcome measures were used for comparison. The acceptability data from the stated willingness-to-pay questionnaire was the primary data source for the economic analysis.

The analysis was a prospective analysis from the healthcare payer's perspective [133]. It included four key components: 1) acceptance of vaccines based on current national data or the willingness-to-pay questionnaire, 2) vaccine dose costs and administration costs for influenza vaccination, 3) influenza illness rates (including hospitalization rates and deaths), and 4) vaccine effectiveness. Outcome measures included coverage improvements, reduction in number of influenza cases, reductions in hospitalizations, reductions in deaths, comparisons of costs to improve coverage, and percent changes in overall vaccination program costs.

A general framework for the economic analysis is shown in Figure A.8, followed by a walk-through of a sample calculation.

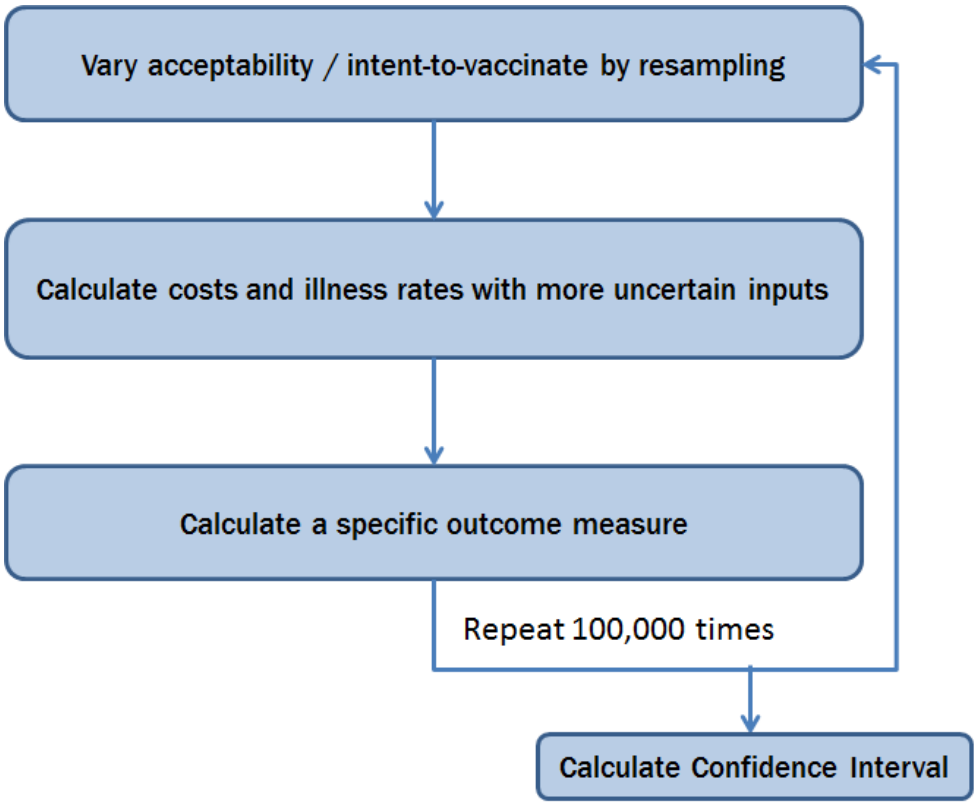

Figure A.8 General Framework for Economic Analysis

A.3.6.1 Sample Calculation

This section includes a sample confidence interval calculation for the reduction in number of cases after introducing a "high-protection" microneedle patch. The economic analysis itself and the calculations for this sample calculation were performed in MATLAB. Most equations shown in this section use the MATLAB language. The first step is to estimate the number of cases of influenza that occur with the current

immunization program. The current coverage level was drawn randomly from the distribution shown in Table 5.3.

baseline_coverage = normrnd(0.455,0.025*0.455); Eq. A.26

The number of people vaccinated before the introduction of microneedles can be calculated using the coverage rate and a population basis size (194 million adults age 18-64 based on the 2010 U.S. Census).

Nvax_IM_before = baseline_coverage*basis; Eq. A.27

The number of influenza cases that occur before the introduction of microneedles depends on the vaccine effectiveness and the influenza attack rate. All vaccinations in this model were assumed to have the vaccine effectiveness given in Table 5.3. The vaccine effectiveness, influenza attack rate, and several other parameters were defined using a triangular distribution. For a random number from a 0-1 uniform distribution, U, a random number from a triangular distribution (with minimum, maximum, and mode of a, b, and c) can be generated with the following formulas [222]:

If $U < \frac{c-a}{b-a}$, $T_{rand} = a + \sqrt{U*(b-a)*(c-a)}$ Eq. A.28

Else, $T_{rand} = b - \sqrt{(1-U)*(b-a)*(b-c)}$ Eq. A.29

Given a random draw for vaccine effectiveness, IM_vax_effectiveness, and a random draw for influenza attack rate, the number of influenza cases expected before the introduction of microneedles is:

cases_before = (basis - Nvax_IM_before*IM_vax_effectiveness)
*influenza_attack_rate; Eq. A.30

Calculating the number of cases expected for a "high-protection" microneedle patch involves estimating the improvement in coverage expected for this scenario and modifying the vaccine effectiveness. The coverage is estimated using resampled data and the techniques described in section A.3.5.1. The subset of participants who normal intend vaccination is resampled (a.k.a. bootstrapped) using the randsample function in MATLAB. Each resampling yields a number of participants who would switch from intramuscular injection to the microneedle patch (Nvax_IM_to_HCW_MN). Similarly, resampling the participants who do not intend vaccination yields a number of participants who would switch from no vaccination to receiving a vaccine patch (Nvax_NO_to_HCW_MN). The number of people choosing each method was defined as:

Nvax_IM_after = Nvax_IM_before - Nvax_IM_to_HCW_MN; Eq. A.31

Nvax_MN_after = Nvax_IM_to_HCW_MN + Nvax_NO_to_HCW_MN; Eq. A.32

For the "high-protection" scenarios, it was assumed the microneedle patch had a higher vaccine effectiveness, 50% closer to 100% effectiveness than the standard vaccine.

MN_vax_effectiveness = IM_vax_effectiveness

+ .5*(1-IM_vax_effectiveness); Eq. A.33

The number of cases expected after the introduction of a microneedle patch could then be calculated as:

cases_after = (basis - Nvax_IM_after*IM_vax_effectiveness -

Nvax_MN_after*MN_vax_effectiveness)*influenza_attack_rate; Eq. A.34

The difference in the number of cases was then calculated as cases_after – cases_before. This calculation represented a single iteration of the model for a single output measure. Because the model relied on uncertain inputs from normal and triangular distributions as well as resampling of data, multiple iterations were used to examine prospective variability in the outcome measures. This model used 100,000 iterations to force the median of separate model runs to differ by less than 5%. With 100,000 iterations, a median and 95% confidence interval (values 2,500 and 97,500 in a sorted array) can be reported for each outcome measure, as shown in Figure A.8.

A.3.7 Theory of Reasoned Action Analysis

In addition to the selected demographic and behavioral correlates, the questionnaire included items designed to measure psychosocial indicators of microneedle acceptability. New scale items were developed based on previous quantitative and qualitative research findings, literature review, and vaccine clinical trial and community experience. [1-4] In addition, psychosocial items were developed for most of the domains based on recommendations by behavioral theorists, guided by the Theory of Reasoned Action and Integrated Behavioral Models [5-9]. A team of investigators with expertise in behavioral researcher and biomedical engineering reviewed the instrument for adequacy of the measures.

The following briefly describes four scale measures developed specifically to assess immunization issues. Each scale item was measured by a 5-point Likert scale (1-strongly disagree to 5-strongly agree), designed to assign meaningful values to an underlying continuum of ratings [10]. These scales were added to the multivariate models as scores based on the average of the component answers of the scale.

Microneedle Use Attitudes. Six items comprised this scale. Four items assessed the benefit of using a vaccine patch compared to existing intradermal injection methods. These included items, "I think that vaccine patches are an improvement over injections," "I prefer a vaccine patch over an injection," "Compared to injections, vaccine patches have benefits that are important to me," and "I like vaccine patches more than injections." In addition, one item compared perceived pain associated with patch use compared to injections. It was phrased, "Putting on a vaccine patch will be less painful than getting an injection." Finally, one item examined participants' attitudes toward injection drawbacks.

The statement read “Compared to vaccine patches, injections have drawbacks that matter to me.”

Behavioral Beliefs. In this seven item scale, we evaluated the extent to which people considered the microneedle innovation to be a reliable and protective mechanism against influenza via two items. They stated, “The vaccine patch can administer flu vaccine reliably” and “I think a vaccine patch could offer as much protection against the flu as an injected vaccine.” Additionally, in four items we explored beliefs associated with its perceived convenience, time savings, self-administration method, and future availability. Participants expressed their level of agreement with these items stating “Putting a vaccine patch on my arm at home is [less safe, as safe, safer] compared to an injection from nurse or doctor,” “A vaccine patch will be more convenient for me than an injection,” “Overall, vaccine patches will help me save time compared to injections,” and “Vaccine patches are easy to use.” The last scale item explore behavioral beliefs associated with ease of use. The statement indicated “I can easily tell if I put a vaccine patch on my arm the right way.”

Normative Approval. Previous studies examining vaccine acceptability have accounted for normative expectations in overall models [11, 12]. Given the extent of evidence suggesting the importance of normative approval as a vaccine decision-making facilitator [2, 4, 13], we developed five items that specifically assessed the expressed or perceived approval of doctors, family, work colleagues, and friends in deciding to use microneedle devices in the future. Questions included “My family will think it’s a good idea if I choose a vaccine patch instead of an injection,” “Important people in my life will approve if I choose a vaccine patch instead of an injection,” “My friends will approve if I

choose a vaccine patch instead of an injection," "My doctor will approve if I choose a vaccine patch instead of an injection," and "Important people in my life would suggest that I get a vaccine patch instead of an injection."

Outcome Evaluation. Issues related to side effects resulting from microneedle use were measured by three items included in this domain. One statement explored participants' perceived likelihood of experiencing bleeding as follows: "A vaccine patch will lead to less bleeding than an injection." Another examined perceived skin damage occurring as a result of its application. It indicated "A vaccine patch will lead to less skin damage compared to an injection." Finally, one item inquired about potential for infection with the statement "A vaccine patch will lead to less risk of infection compared to an injection."

A.3.7.1 Statistical Analysis

SAS version 9.3 was used for analyses (SAS Institute Inc, Cary, NC, USA). Descriptive statistics and cross-tabulations were generated for variables of interest. Bivariate correlations were also generated to explore key relationships. An exploratory factor analysis was conducted using SPSS version 20.0 and resulting scale reliability estimates were generated. The lower bound for item extraction was determined based on resulting values of ≥ 0.50. We determined a Cronbach alpha reliability estimate of ≥ 0.70 would support reliability of each subscale [14]. Multivariate logistic regression models were used to analyze the independent contributions of variables. Significant independent predictors of outcomes were assessed at $\alpha=0.05$ levels.

A.3.7.2 Factor Analysis and Internal Consistencies

With 21 questionnaire items, we conducted an exploratory principal components factor analysis using Varimax rotation method that resulted in a 4-factor solution (65.54% cumulative variance). The first extracted factor, "Microneedle Attitudes," comprised the largest portion of the variance (37.23%) with an initial Eigenvalue total sum of square loading totaling 7.82. Following rotation, its contribution to the cumulative variance resulted in 20.39% of the total variance value of 65.54%. The "Behavioral Beliefs" component added the second largest contribution to the overall variance with a final rotated value of 18.52% of the variance thereby resulting in 38.91% with consideration of the attitudinal component. "Subjective norms" provided an additional 14.82% of the total variance as the third factor and "Outcome Evaluations" contributed the final 11.82% of the cumulative percentage accounted for by these factors (65.43%). The internal consistencies achieved on the four scales demonstrated a high level of reliability. "Microneedle Attitudes" resulted in the highest alpha score of 0.904. This was followed by "Normative Approval" ($\alpha = 0.845$) and "Behavioral Beliefs" ($\alpha = 0.845$) and "Outcome Evaluations" ($\alpha = 0.806$).

A.3.7.3 References for Theory of Reasoned Action Analysis

1. Hutchins, S., et al., *Protection of racial/ethnic minority populations during an influenza pandemic.* Am J Public Health, 2009. **99**(Suppl 2): p. S261-70.
2. Salmon, D., et al., *Knowledge, attitudes, and beliefs of school nurses and personnel and associations with nonmedical immunization exemptions.* Pediatrics, 2004. **113**(6): p. e552-e559.
3. Allred, N., et al., *Parental vaccine safety concerns: Results from the National Immunization Survey, 2001-2002.* Am J Prev Med, 2005. **28**(2): p. 221-224.
4. Smith, P., et al., *Association between health care providers' influence on parents who have concerns about vaccine safety and vaccination coverage.* Pediatrics, 2006. **118**: p. e1287-e1292.

5. Ajzen, I. and M. Fishbein, *Understanding attitudes and predicting behavior*. 1980, Englewood Cliffs, NJ: Prentice-Hall.
6. Fishbein, M. and I. Ajzen, *Belief, attitude, intention, and behavior*. 1975, Menlo Park, CA: Addison-Wesley.
7. Montano, D.E. and D. Kasprzyk, *Theory of Reasoned Action, Theory of Planned Behavior, and The Integrated Behavioral Model*, in *Health Behavior and Health Education: Theory, Research, and Practice*, K. Glanz, B. Rimer, and K. Viswanath, Editors. 2008, Jossey-Bass: San Francisco, CA. p. 67-96.
8. Green, M.S., *Compliance with influenza vaccination and the health belief model.* The Israel Medical Association journal : IMAJ, 2000. **2**(12): p. 912-3.
9. Nexoe, J., J. Kragstrup, and J. Sogaard, *Decision on influenza vaccination among the elderly. A questionnaire study based on the Health Belief Model and the Multidimensional Locus of Control Theory.* Scandinavian journal of primary health care, 1999. **17**(2): p. 105-10.
10. Meyers, L.S., G. Gamst, and A.J. Guarino, *Applied Multivariate Research: Design and Interpretation*. 2006, Thousand Oaks: Sage Publications. 722.
11. Fishbein, M., et al., *Factors influencing behavior and behavior change*, in *Handbook of health psychology*, A. Baum, T.A. Revenson, and J. Singer, Editors. 2001, Lawrence Erlbaum Associates: Mahwah, New Jersey. p. 3-14.
12. Frew, P.M., et al., *An Extended Model of Reasoned Action to Understand the Influence of Individual- and Network-Level Factors on African Americans' Participation in HIV Vaccine Research.* Prevention Science, 2009. **in press**.
13. Shui, I., et al., *Factors influencing African-American mothers' concerns about immunization safety: a summary of focus group findings.* J Natl Med Assoc, 2005. **97**(5): p. 657-66.
14. Cronbach, L., *Coefficient alpha and the internal structure of tests.16(3).* Psychometrika, 1951. **16**(3): p. 297-334.

APPENDIX B

FORMS USED IN HUMAN STUDIES

For the human studies described in Chapters 4 and 5, this appendix contains protocols, consent forms, assent forms, and data sheets.

B.1 Consent Form for Insulin Study

Emory University School of Medicine
Informed Consent to be a Research Subject & Authorization Form

TITLE: Transdermal Delivery of Insulin through Microneedles in Children with Type 1 Diabetes.

PRINCIPAL INVESTIGATOR and SPONSOR: Eric I. Felner, M.D.

STUDY SUPPORTER: Thrasher Foundation

If the patient is under 18 years of age, the remainder of the consent addresses the parent or legal guardian and "you" means "your child."

INTRODUCTION:
You are being asked to be in a medical research study. This form is designed to tell you everything you need to think about before you decide to consent (agree) to be in the study or not to be in the study. It is entirely your choice. If you decide to take part, you can change your mind later on and withdraw from the research study. The decision to join or not join the research study will not cause you to lose any medical benefits. If you decide not to take part in this study, your doctor will continue to treat you.

- Please carefully read this form or have it read to you
- Please listen to the study doctor or study staff explain the study to you
- Please ask questions about anything that is not clear
- Feel free to take home an unsigned copy of this form and take your time to think about it and talk it over with family or friends

If you agree to join this research study, you will receive a copy of this consent form with your signature and the date, to keep. Do not sign this consent form unless you have had a chance to ask questions and get answers that make sense to you. Nothing in this form can make you give up any legal rights. By signing this form you will not give up any legal rights.

PURPOSE:
The purpose of this project is to see if an insulin pump can deliver insulin to you through a very small needle that is much smaller than the needle you use with your pump. At its tip, this small needle will be smaller than the width of a hair. This small needle is a microneedle. We are trying to enroll patients (greater than 6 years of age) with diabetes who wear an insulin pump. We are trying to determine if this small needle may be better than the needle you are currently using with your pump. We will test if the microneedle delivers insulin as well as a regular needle and if the microneedle causes less pain or irritation than a regular needle. We are performing these tests for bolus (before a meal) and basal delivery. Dr. Felner will inform you of the type of insulin delivery he would like you to receive. One of the investigators will insert the insulin pump catheter or microneedle under the medical supervision of Dr. Eric Felner. The insulin pump catheter or microneedle connects to an infusion pump that will administer insulin. The microneedles are experimental and do not have FDA approval.

We hope to enroll 20 subjects in the microneedle study at Emory.

PROCEDURES:
Participation involves 2 visits to the Emory Children's Center (ECC). Dr. Felner will decide if you have the bolus or basal tests. Each visit lasts for about 5 hours and will be 1-2 weeks apart. You will arrive for all visits by 8 AM. You will not eat or drink after 10 PM the night before the visit. When you arrive to the clinic, a nurse will place an intravenous catheter (IVC) in a vein in your wrist or arm and remove your insulin pump. The day before each clinic visit, Dr. Felner will call you and set your pump's basal rate to help you have stable sugar and insulin levels the day of the visit. At each visit, the nurse will collect blood from your IVC every 15 minutes for a total of 4-hours. The blood will allow us

to measure your sugar and insulin levels during the study. The nurse will collect no more than 120 ml (about 25 teaspoons) of blood from you at each visit. At the time of each blood collection from your IVC, the nurse may also collect a drop of blood by finger stick.

If you are a pubertal female, Dr. Felner will carry out a pregnancy test on you before the study begins. This involves collecting a small amount of urine or a drop of blood. Dr. Felner will tell you the results of this pregnancy test. If you are pregnant, you will not be able to participate in this study.

Upon arrival to the ECC, Dr. Felner will decide, by chance, the delivery device used at the first visit. Dr. Felner will test the other device at the next visit.

Specific Visits: Bolus Delivery

Visit #1
Upon arrival to the ECC, you will remove your insulin pump. The nurse will place an IVC in your arm. One of the investigators will insert an insulin pump catheter or a microneedle in your abdomen. The insulin pump catheter and microneedle connect to an infusion pump through clear tubing. Dr. Felner will set the infusion pump to give you the amount of Lispro insulin (based on your insulin to carbohydrate ratio) to cover a 75 gram, standard, mixed meal including 75-grams of carbohydrates. You will not eat or drink for the 4-hours of insulin delivery. Insulin will be infused in less than 5 minutes, and the catheter OR microneedle will be removed after insulin delivery is complete and Dr. Felner will assess the insertion site for skin irritation. One of the investigators will ask you to rate the pain you have with insertion of the delivery device and with insulin delivery through the device. We will ask you to use a device that looks like a ruler to show the pain you feel from the needle. You will move a pointer on the device to show if you feel no pain, some pain or a lot of pain from the needle. Once the insulin has been delivered and you have rated the pain, you will eat the 75-gram carbohydrate meal. The nurse will collect blood from your IVC over the next 4-hours. After the last blood sample is collected, the nurse will remove the IVC and you will reinsert your insulin pump catheter and restart your pump.

Visit #2:
The same procedure occurs as in Visit #1 but with the other delivery device.

If your sugar level drops below 60 mg/dL, you will drink juice until your sugar is above 80 mg/dL. If you are unable to swallow, Dr. Felner will inject a sugar liquid through the IVC until you can swallow. Blood testing will stop if your sugar drops below 60 mg/dL at any time during the visit.

Specific Visits: Basal Delivery

Visit #1:
Upon arrival to the ECC, you will remove your insulin pump. The nurse will place an IVC in your arm. One of the investigators will insert an insulin pump catheter or a microneedle in your abdomen. The insulin pump catheter and microneedle connect to an infusion pump through clear tubing. Dr. Felner will set the infusion pump to give you the amount of Lispro insulin (based on your home basal rate) that you receive from 8 AM - 12 PM. You will not eat or drink for the 4-hours of insulin delivery. Shortly after catheter OR microneedle insertion, one of the investigators will ask you to rate the pain you have with insertion of the delivery device. We will ask you to use a device that looks like a ruler to show the pain you feel from the needle. You will move a pointer on the device to show if you feel no pain, some pain or a lot of pain from the needle. The nurse will collect blood from your IVC over the next 4-hours. Once the insulin has been completely delivered (4-hours), one of investigators will ask you to rate the pain you have with insulin delivery during the 4-hours. Once the delivery and pain assessment is complete, Dr. Felner will remove the catheter OR microneedle and assess the insertion site for skin irritation. After the last blood sample is collected, the nurse will remove the IVC and you will reinsert your insulin pump catheter and restart your pump.

Visit #2

The same procedure occurs as in Visit #1 but with the other delivery device.

If your sugar level drops below 60 mg/dL, you will drink juice until your sugar is above 80 mg/dL. If you are unable to swallow, Dr. Felner will inject a sugar liquid through the IVC until you can swallow. Blood testing will stop if your sugar drops below 60 mg/dL at any time during the visit.

RISKS AND DISCOMFORTS ASSOCIATED WITH PARTICIPATION:

Every study involves some risk. There may be side effects from this study that are not known at this time. The most common risks and discomforts expected in this study are pain, soreness and bruising at the site of needle insertion. There is a small chance that an infection may occur at the site of insulin injection. We will take precautions to prevent infections. We use sterile materials and wipe the skin with alcohol before attaching microneedles. There is a small chance that you get a small scratch or bruise at the site of the attachment site. There is also a small chance that the microneedle breaks or remains in the skin. If the microneedle breaks off in your skin, a granuloma may form. This is a hard spot formed within your skin that can be unpleasant, but not life threatening. The granuloma may take up to several months to appear but a physician can remove it. If you develop a granuloma, you should notify the Contact Persons listed at the end of this form. There is some chance that microneedles made from nickel can cause redness, itchiness, or blisters (e.g., like a mosquito bite). Such a reaction usually clears up once the investigator removes the nickel from the skin. You will be informed if microneedles made from nickel will be used. An IVC will be placed in a vein in your wrist or arm. Rare but possible risks include infection at the site of needle insertion when the blood samples are collected. Occasionally, fainting or lightheadedness may occur. Only experienced personnel will draw blood samples. Finger pressure and/or ice packs will be applied for at least 5 minutes after your blood is collected if needed.

There is also a chance that too much or too little insulin will be administered to you. We will recognize that with our frequent capillary glucose (finger stick) monitoring and/or by your behavior. If too little insulin is given, we will give you insulin through a subcutaneous injection, if needed. If too much insulin is given, we will provide you with juice by mouth or a sugar solution by intravenous injection, if needed. If too much insulin is given, you may develop dizziness or lightheadedness, possibly loss of consciousness, and there is a remote possibility of death. It is, however, unlikely that too much or too little insulin will be given such that serious side effects or risks will occur.

NEW INFORMATION:

If new findings become available that affect your desire to continue the study, we will inform you of them. We will give you additional reading materials on any new risk if one develops.

BENEFITS ASSOCIATED WITH PARTICIPATION:

There are no direct benefits of this research to you. There may be a benefit to society from your participation. If successful, this project could make the administration of insulin or other drugs possible without the use of a hypodermic needle.

REIMBURSEMENT FOR PARTICIPATION:

You will receive $150 total for your time, inconvenience, travels, and parking for the 2 visits involved in this study. You will receive $75 for each completed clinic visit. If you do not finish the study, you will be paid for the visits you have completed.

ALTERNATIVES TO PARTICIPATION:

The only alternative is not to participate in this study. If you do not participate in the study, your usual medical care will not be affected in any way.

CONFIDENTIALITY:

Certain offices and people other than the researchers may look at your medical charts and study records. Government agencies, Emory employees overseeing proper study conduct may look at your study records. Study sponsors may also look at your study records. These offices include the Food and Drug Administration, the Office for Human Research Protections, the sponsor, the Emory Institutional Review Board, the Emory Office of Research Compliance and the Office for Clinical Research. Emory will keep any research records we produce private to the extent we are required to do so by law. A study number rather than your name will be used on study records wherever possible. Your name and other facts that might point to you will not appear when we present this study or publish its results. Study records can be opened by court order or produced in response to a subpoena or a request for production of documents.

If you are or have been an Emory Healthcare patient, you have an Emory Healthcare medical record. If you are not and have never been an Emory Healthcare patient you do not have one. Please note that an Emory Healthcare medical record will not be created for you just because you are in this study.

To better protect the confidential nature of your research information, the results from these study tests and procedures should not be included in any medical record you have: insulin and blood glucose assays

These research results will be kept by the researchers only in a research record. The researchers will take steps to make sure that these results are not placed in your Emory Healthcare medical record. The results will not be made available to any other healthcare providers who may be giving you treatment. It will be up to you to let your healthcare providers know that you are in a research study.

Other useful study results that are not on this list will be placed your Emory Healthcare medical record. Anyone who has access to your medical record will be able to have access to all results that are placed there. Emory Healthcare may use these results in caring for you. The confidentiality of the study information in your medical record will be protected by laws like the HIPAA Privacy Rule. On the other hand, some state and federal laws and rules may not protect the research information from disclosure.

Emory does not control results from tests and procedures done at other places. So these results would not be placed in your Emory Healthcare medical record and they will not likely be available to Emory Healthcare to help take care of you. Emory also does not have control over any other medical records that you may have with other healthcare providers. Emory will not send any test or procedure results from the study to these providers. So if you decide to be in this study, it is up to you to let them know.

Some tests and procedures that may be done during this study will be reviewed only for research purposes, not for your healthcare purposes. These results will not be reviewed to make decisions about your personal health or treatment. The specific tests or procedures, if any, would be reviewed only for research purposes include: insulin and blood glucose assays

We encourage you to let your health care provider know if you decide to take part in this study. That way they can have extra information that can help them to make decisions about your health care.

We will put a copy of your signed informed consent form for the Research Study into any medical record that you may have with Emory Healthcare facilities. Laboratory and medical procedure results received from Emory Healthcare facilities may also be placed in any medical record that you have with Emory Healthcare facilities.

COMPENSATION & TREATMENT FOR INJURY:

If you get ill or injured from being in this study, Emory would give/arrange for you to have urgent health care. Here we explain who would pay for this health care:

Would Emory Pay? Emory has not set aside any funds to pay for urgent health care. Also, Emory has not set aside any funds to pay you if you become ill or injured from being in this study. The only exception to this policy is if it is proven that the negligence of an Emory employee directly caused your injury or illness. "Negligence" means the failure to follow a standard duty of care.

Would the Study Sponsor or the Thrasher Foundation Pay? The Study Sponsor and the Thrasher Foundation have not set aside any funds to pay for urgent health care. In addition, the Study Sponsor has not set aside any funds to pay you if you become ill or injured from being in this study.

If you believe you have been injured by this research, you should contact Eric Felner, M.D. at (404) 727-9811.

COSTS OF PARTICIPATION:
There are no costs to you. Costs for your regular medical care (office visits, insulin, and diabetes supplies) that are not related to this study will be your responsibility or the responsibility of your insurance carrier.

WITHDRAWAL FROM THE STUDY:
You have the right to leave a study at any time without penalty. For your safety, however, you should consider the study doctor's advice about how to go off the study device. If you leave the study before the final planned study visit, the study doctor may ask you to have some of the final steps done.

The Study Doctor(s) may stop your participation in the study without your consent at any time if:
- The Study Doctor(s) believes it is in your child's best interest;
- You significantly fail to follow the study's appointments and procedures;
- You develop any health condition that further study participation aggravates;
- You develop any health condition that prevents you from fully completing the study; or
- You have a serious problem during any of the procedures.

If you withdraw from the study, you must notify Dr Eric Felner at (404) 727-9811. If you withdraw, your usual medical care will not be affected in any way. In the event that you withdraw from the study, you may request that all collected data be destroyed. The Study Doctors will send a written notification that the data was destroyed at your request.

HEALTH INSURANCE PORTABILITY and ACCOUNTABILITY ACT (HIPAA)

Disclosure of your Health Information In Accordance with the Health Portability and Accountability Act (HIPAA):

PHI is a term we use for protected health information. PHI are any facts about your health that could tell someone who you are. "Researchers" are what we call the people who are conducting the study. They may need to look at your medical and study records that contain PHI. Government agencies also may need to look at your records. They make rules and policies about how research is done. They include the Office for Human Research Protections and the Food and Drug Administration. Sponsors who pay for the study also have the right to review records. So does the Emory University Institutional Review Board (IRB). This could include an IRB at another site if the study is being done at more than one place. Your PHI may be disclosed if a court of law should order it.

We will not use or disclose your records in any ways other than the ways we describe in this form. We will keep your records private to the extent allowed by law. We will do this even if outside review of your records occurs. We will use a study number or other code rather than your name on study records where we can. Your name and other facts that might point to you will not appear when we present this study or publish its results.

A federal law now protects the privacy of your PHI. This law is the Health Insurance, Portability, and Accountability Act (HIPAA). That law says we must tell you what we will use your PHI for and how we will use and disclose it. We give you those facts about your PHI in this section. It will tell you:

1. What PHI the Researchers will look at.
2. Who will collect your PHI.
3. Who will use your PHI.
4. With whom your PHI will be shared and why it is shared each time.
5. The date or event, if any is set, after which we will not use or disclosure your PHI.
6. Your rights under HIPAA to ask us not to use your child's PHI any more.

You may choose to join in this research. If you do, you will agree to let the Researchers and any other persons, companies or agencies described below use and share your PHI for the study in the ways that are set forth in this section. Please review this section carefully.

What PHI will the Research Team Use?
The Researchers will look at information that identifies you such as your name, patient identification number, and medical record number. The Researchers will also look at any results from your laboratory tests. They may record this identifiable information in your research file. In addition, if you have a bad outcome or 'adverse event,' the Researchers may also look at your entire medical record.

What Health Information that Identifies You that Will be Used or Disclosed? The Information Users may use or disclose the following health information about you: the entire medical record including birth date, diseases, treatments, results of blood tests, and study results.

Who will collect the PHI?
Dr. Felner and other members of the research team will collect and copy the PHI described above. If they share any of the PHI with other persons (described later on in this section), the Researchers will also be responsible for making these disclosures.

Who will Use the PHI; With Whom will it be Shared; and For What Purpose(s) Will it be Used or Shared:

Person/Entity	Purpose
Dr. Felner and investigators	To conduct the study entitled "Transdermal Delivery of Insulin through Microneedles in Children with Type 1 Diabetes."
Governmental Agencies with oversight over the research being conducted, including the FDA and OHRP.	To monitor safety, efficacy and compliance with applicable laws and regulations.
Emory University personnel, committees and departments charged with oversight of research, including the IRB.	To monitor safety and compliance with applicable laws, regulations and University policies and procedures.
Statisticians hired by the study sponsor.	To perform data analysis.
Researchers working on this study.	To assist in the conduct of this study; for future research related to this study; and to report problems ("adverse events") that occur

	during the study.

Your Right Under HIPAA to Revoke Authorization and Ask us Not to Use Your PHI any More:
It is your free choice to give the Researchers your OK to use and share your PHI. The term for this OK is called your "authorization." At any time, you may take back your authorization for the Researchers to use and share your PHI. The term we use for taking back your authorization is "revoke." Revoking your authorization means the Researchers may no longer be able to treat your child as they do now because you are in the study. Revoking your authorization will not have a bad effect on your current or future health care. Revoking your authorization also does not involve a penalty and it does not involve the loss of any benefits, which you could get otherwise. It is a simple process to revoke your authorization for us to use your PHI. You may do this by completing and signing what we call a "revocation letter." We will give you a copy of that letter along with your copy of this Combined Informed Consent/HIPAA Authorization form. You would fill it out and sign it if you choose to revoke your authorization. Then you would give it to the researchers. The Researchers will give you another copy at any time you want one. You must make a written request to revoke your authorization to use your PHI. We will act at once if we get a letter from you revoking your authorization to use your PHI.

Other Items You Should Know:
HIPAA only applies to people or organizations that are health care providers, health care payers or healthcare clearinghouses. HIPAA may not apply to all Information Users. If HIPAA doesn't apply to an Information User, then that User doesn't have to follow HIPAA requirements when it uses or discloses your health information.

You do not have to sign this authorization form, but if you do not, you may not participate in the Research Study or receive research-related treatment. You may still receive non-research related treatment.

We will put a copy of your signed informed consent form for the Research Study and your signed HIPAA Authorization form into any medical record that you may have with Emory Healthcare facilities. Laboratory and medical procedure results received from Emory Healthcare facilities may also be placed in any medical record that you have with Emory Healthcare facilities.

If the Research Study involves medical treatment, then, in order to maintain the integrity of the research study, you generally will not have access to your personal health information related to this Research Study until the study is complete. When the study is complete, then, at your request, you may generally have access to any of your personal health information related to the research that makes up a part of the medical information and/or other records that your health care providers use to make decisions about you. If access to this information is needed before the end of the Research Study for your treatment, then the information may be provided to your physician.

If your identifying information is removed from your health information, then the information that remains will not be subject to this authorization or covered by HIPAA, and it may be used or disclosed to other persons or organizations, and/or for other purposes.

Expiration Date or Event:
The Researchers will add your PHI to a database that they are putting together for research purposes. This authorization will expire when the research study ends, which will occur after 20 subjects have been enrolled and completed the protocol.

Signature and Date:
The researchers will ask you to sign and date this form. One of the investigators will place a copy of this form in your medical record(s).

PHI May be Re-disclosed:

If we disclose or reveal your PHI to one of the other parties described above, they might further disclose your PHI to another party. If your PHI is further disclosed, the information is no longer covered by HIPAA. Your name and other facts that might point to you will not appear when we present this study or publish its results.

QUESTIONS:

Contact Dr. Eric Felner at (404) 727-9811:

- if you have any questions about this study or your part in it,
- if you feel you have had a research-related injury or a bad reaction to the study drug, or
- if you have questions, concerns or complaints about the research

If you have any questions or concerns about your rights as a participant in this research study, you may contact the Emory Institutional Review Board at 404-712-0720 or 877-503-9797.

CONSENT OF SUBJECT

I have read or, one of the investigators has read to me, a description of the study as outlined above. One of the investigators explained the study to me and answered all the questions I have at this time. An investigator informed me of potential risks, discomforts, side effects, adverse reactions, and possible benefits of the study.

I freely volunteer to participate in the study. I do not have to take part in this study. My refusal to participate will involve no penalty or loss of rights to which I am entitled. I am free to later withdraw my consent and discontinue participation in this study at any time. If I refuse to participate or later withdraw from the study, it will not affect my subsequent medical care. By signing this consent form, I have not given up any of my legal rights.

You will receive a copy of this consent form. Your signature below indicates that you consent to volunteer for this study.

_____ _____
Subject name (*printed*) Subject code #

_____ _____ _____
Signature of Subject or Parent/Legally Authorized Representative Date Time

If Representative, Relationship to Study Subject: _____

_____ _____ _____
Signature (assent of subject, age 17) Date Time

_____ _____ _____
Signature of Person Conducting Informed Consent Discussion Date Time

B.2 Assent Form for Insulin Study

Study No.: IRB00024833 | **Emory University IRB** IRB use only | Document Approved On: 1/19/2011 Project Approval Expires On: **1/18/2012**

Documentation of Assent from Pediatric Subjects

Subject age:_____years.

For subjects in this study who are minors, one of the following Pediatric Assent sections must be satisfied. Place a checkmark beside the method used.

1._____(< 6 years) NO ASSENT REQUIRED

2._____ (ages 6-10) VERBAL ASSENT

The study and treatment has been explained to this child in an age-appropriate manner. The child has asked questions, verbalizes understanding of the information, and provides verbal assent.

_____ _____ _____
Person Soliciting Assent Date Time

3._____ (ages 11-16) WRITTEN ASSENT See attached Written Assent document
.

4._____ (age 16-17) READ/SIGN MAIN CONSENT DOCUMENT WITH GUARDIAN

5._____ (any age) UNABLE TO PROVIDE ASSENT

In my opinion, this child cannot give informed assent.

Reason(s):_____

_____ _____ _____
Person Soliciting Assent Date Time

_____ _____ _____
Signature of Investigator Date Time

Ver. 3/26/2010 Page 1 of 2

Emory University
Assent Form

TITLE: *Transdermal Delivery of Insulin through Microneedles in Children with Type 1 Diabetes*

PRINCIPAL INVESTIGATOR:
Eric Felner, M.D.

1. What are we asking you to do?
You are being asked to volunteer for a research study because you have diabetes and use an insulin pump. The purpose is to see if there is a less painful way to insert your pump and deliver insulin.

2. If you agree to participate:
You will come to the clinic 2 times for the study. At each visit, blood will be collected from you. On 1 of the visits, one of the investigators will insert your regular pump needle to you, just like you and your parents normally do at home. At the other visit, a TINY needle device will be attached to your skin by one of the investigators. For both visits, a pump will deliver insulin to you. Dr. Felner and his team will compare the blood sugar and insulin results from the TINY needle device with those from your regular pump needle system.

3. Why are we asking you to participate?
We are asking you to volunteer because you have diabetes and use an insulin pump. We want to find out if these TINY needles are less painful than the needle that you are using with your pump.

4. How will this study help you?
If you participate, it is possible that receiving insulin may be less painful than with your pump needle.

5. What are the risks?
There is a small chance that you will get a bruise where the needles are attached to your skin.

6. Do you have to participate in the study?
You can refuse to be in this study, and your parent(s), legal guardian(s) or your doctor cannot force you to participate. You can stop being in the study at any time.

7. What do you get for participating in the study?
If you agree to volunteer, you will receive $75 for each study visit, for a total of $150.

8. Signatures
Your signature on this form indicates that you have read this form and had the opportunity to ask questions about the study. If you are willing to volunteer for this research study, please sign below.

Signature of Participant (ages 11-17): _____ Date/Time: _____

☐ **Verbal assent only**

Person Soliciting Assent: _____ Date/Time: _____

SUBJECT ID# _____

INSULIN HOLLOW MICRONEEDLE CLINICAL STUDY

EMORY PROTOCOL – IRB ID# 1348-2005

PI: Eric Felner, MD, MSCR

FORMS SIGNED & COMPLETED:

_____Informed Consent

_____HIPPA Form

_____Documentation of Assent from Pediatric Subjects

_____Written Assent Form

INCLUSION CRITERIA
(Enter subject data values for each criterion. If criteria not met, subject should be excluded):

DOB:_____Age (≥ 10 years)

_____# of years with Type 1 diabetes (≥ 2 years)

_____Type of pump and insulin used (conventional FDA pump and lispro)

_____# of years on pump (≥ 1 year)

_____Mean HbA1C level (< 8.5 % for past year)

_____ BMI (≤ 85th percentile for age) : Height _____cm Weight _____kg

SUBJECT ID# _____

EXCLUSION CRITERIA:
(Check if criteria fulfilled. If subject does not full ANY of the following criteria, exclude from study):

_____Subject does not have type 2 diabetes

_____Subject does not have acanthosis nigricnas

_____Subject does not have clinically significant major organ disease

_____Subject is not on glucocorticoid therapy

_____Subject does not have insulin requirement > 150 U/day

_____Subject does not have illness during study

_____Subject does not have cognitive impairment (IQ<85 or > 2 grades behind age-appropriate grade)

_____Subject (if female) is not pregnant *(confirmed by test)* or breastfeeding

IV CATHETER PLACEMENT:

VAS Pain Score: _____

COMMENTS/NOTES:

SUBJECT ID# _____

VISIT #1 (MN or SCC)

VAS Pain Score: _____ (Insertion)

VAS Pain Score: _____ (Delivery)

Date: _____

Subject ID#: _____

Units of Insulin Delivered: _____ Volume of U-50 Delivered: _____

Delivery Rate: _____ Catheter/MN Length/Depth: _____mm

Time Point (min)	Real Time (AM)	Capillary Glucose (mg/dL)	Plasma Glucose (mg/dL)	Plasma Free Insulin (μU//mL)	Edema/ Erythema Bleb Dia	Comments
Pump Off						**Fasting:** Yes/No
Pre:						**Meal after Bolus:** Yes/No
Pre:						
Pre:						***Delivery Comments:***
0						**Bleb Observed:**
15						_____
30						
45						**Skin Visual Obs:**
60						_____
75						
90						
105						
120						
150						
180						
210						
240						

B.4 Consent Form for Self-Vaccination Study

Subject code # _____

Georgia Institute of Technology
School of Chemical and Biomolecular Engineering
Informed Consent Form

PROTOCOL TITLE: Microneedles for transdermal delivery

PRINCIPAL INVESTIGATOR:
Mark Prausnitz, Ph.D.
Professor of Chemical and Biomolecular Engineering, Georgia Tech

STUDY SUPPORTER(S):
National Institutes of Health
Georgia Institute of Technology

INTRODUCTION/PURPOSE: You are being asked to volunteer for a research project. For this project, there will be up to 200 participants. The purpose of this project is to assess the potential for microneedles to be used to administer medicine and make physiological measurements. Microneedles are very small needles that are either too small to see or are just barely visible. We have prepared small devices that contain one or more microneedles that will be applied to your skin and the microneedles will pierce the very surface of your skin. Although these microneedles are extremely short, they are long enough to cross the skin's thin outer barrier and thereby enable delivery of medicines into the body and withdrawal of molecules out of the body for physiological measurements. Successful development of microneedles could enable, for example, painless administration of drugs that normally require hypodermic shots and physiological measurements that normally require drawing blood.

Participants must meet certain criteria. For this study, the following criteria apply.

☐ Participants must be healthy adults.

☐ Participants must not have diseased or otherwise abnormal skin, subject to the investigator's discretion.

☐ Participants must have no disease known to affect nerve function or perception of pain.

☐ Participants must not have known allergy to nickel.

☐ Participants must not have known allergy to gentian violet, Betadine (povidone-iodide 10%), Liquid Band-Aid, fluorescein or other FDA-approved treatments for minor skin injuries.

☐ For studies involving fluorescein, participants must not be pregnant or nursing.

_____ Initial here if you meet all of the criteria checked off above.

PROCEDURE: In this study, one or more procedures will be carried out. You may not be allowed to watch the procedures during part or all of the study because watching could influence your responses for the study. These procedures will be performed by Mark Prausnitz, Ph.D. or members of his research group at the Georgia Institute of Technology. All materials used in these procedures will be sterilized. Your skin may be wiped with an alcohol swab before and

after the study procedure. Participation in this study will require up to _____ hours of your time and _____ visit(s).

You are being asked to participate in the following procedures that have been checked below (i.e., you are not being asked to participate in those procedures listed below that are not checked). Although you are being asked to participate in a one or more procedures, they are all part of a single, integrated study and will all be carried out during the same visit. You will need to agree to participate in all of the checked procedures in order to participate in this study. If you only agree to participate in some of the checked procedures, you will not be able to participate in this study.

☐ **Microneedle device applied to skin**

_____ Initial here if you will participate in this procedure.

This is the main procedure we are studying. For this procedure, microneedle devices will be applied at up to _____ skin sites on your forearms or back of your hands so that the microneedles penetrate into your skin. They will be left in place for up to _____ seconds/minutes. Each device will be a small, hand-held object that contains one or more microneedles. The microneedles may be solid or they may be hollow and made of metal, plastic or glass. Unless you are told otherwise (below), the microneedles will not be used to deliver any medicine or other material into your body and will not be used to withdraw material out of your body for physiological measurements. Some of the devices may be "placebo" devices, meaning that they look like microneedles devices, except they do not have any microneedles. Skin sites may be labeled by marking with ink. The ink will be removed with an alcohol wipe.

☐ **Microneedle device applied to your skin by you**

_____ Initial here if you will participate in this procedure.

For this procedure, you will apply microneedle devices at up to _____ skin sites on your forearms or back of your hands so that the microneedles penetrate into your skin. They will be left in place for up to _____ seconds/minutes. You will receive written instructions for how to apply microneedles devices to your skin. Each device will be a small, hand-held object that contains one or more microneedles. The microneedles will be solid and made of metal. Unless you are told otherwise (below), the microneedles will not be used to deliver any medicine or other material into your body and will not be used to withdraw material out of your body for physiological measurements. Some of the devices may be "placebo" devices, meaning that they look like microneedles devices, except they do not have any microneedles. Skin sites may be labeled by marking with ink. The ink will be removed with an alcohol wipe.

☐ **Hypodermic needle, acupuncture needle or lancet inserted into skin**

_____ Initial here if you will participate in this procedure.

We are carrying out this procedure to compare to microneedles. For this procedure, medical needles (i.e., hypodermic needles, acupuncture needles or lancets) will be inserted at up to _____ skin sites on the forearms or back of your hands. They will be left in place for up to _____ seconds/minutes. Unless you are told otherwise (below), the medical needles will not be used to deliver any medicine or other material into your body

and will not be used to withdraw material out of your body for physiological measurements. Skin sites may be labeled by marking with ink.

☐ **SonoPrep® applied to skin**

_____ Initial here if you will participate in this procedure.

We are carrying out this procedure to compare to microneedles. For this procedure, an FDA-approved device called SonoPrep® will be applied at up to _____ skin sites on the forearms or back of your hands. The device will be left in place for 30 seconds, during which time it will apply a low level of ultrasound energy, which will temporarily make your skin more permeable. Unless you are told otherwise (below), the device will not be used to deliver any medicine or other material into your body and will not be used to withdraw material out of your body for physiological measurements. Skin sites may be labeled by marking with ink.

☐ **Saline injection**

_____ Initial here if you will participate in this procedure.

Saline will be injected into your skin using hollow microneedles and/or standard hypodermic needles. The hypodermic needles will be between 25 and 30 gauge and between 3/8 to 5/8 of an inch in length. Saline may also be injected beneath your skin or into your muscle using hypodermic needles. Hypodermic needles will be between 20 and 30 gauge and between ½ and 1½ inches long. The hypodermic needles will be attached to a syringe and the microneedles will be attached to a syringe or pump that will inject up to 1 milliliter (i.e., equal to about 20 drops) of sterile saline (0.9% sodium chloride for injection, USP grade) into your skin for up to 5 minutes during injection using microneedles or up to 10 seconds during injection using hypodermic needles. The investigator may measure the pressure applied by the syringe or pump and the time it takes to inject the saline. Each microneedle and hypodermic needle will be removed from your skin immediately after the injection is complete.

In this study you will receive the following injections. The number of times you will receive each injection is also indicated

- ☐ Injection of saline into your skin using microneedles (____ injections will be performed).
- ☐ Injection of saline into your skin using hypodermic needles (____ injections will be performed).
- ☐ Injection of saline beneath your skin using hypodermic needles (____ injections will be performed).
- ☐ Injection of saline into your muscle using hypodermic needles (____ injections will be performed).

☐ **Skin fluid withdrawal**

_____ Initial here if you will participate in this procedure.

A device that applies suction will be applied to ____ skin sites to withdraw up to 0.01 ml (which is equal to a few drops) of fluid from the skin. This will involve applying to your skin a small funnel-like apparatus that is connected to tubing and a pump. This device will apply mild suction to your skin for up to 5 min at each site. The fluid collected in this way may be clear fluid from the skin or it may contain blood. In addition, up to 0.01 mL of blood may be collected by a lancet puncture for ___ skin sites. The collected fluid and blood may be assayed for glucose (i.e., sugar) content and may be weighed. The fluid and blood will not be analyzed for other contents and will be discarded after the glucose and weight assays are complete.

☐ **Insertion force measurement**

_____ Initial here if you will participate in this procedure.

When the microneedles and/or medical needles are inserted into your skin as described above, they will be inserted using a device that will measure the position of the needles, the force applied to the needles and the electrical resistance of your skin before, during and after insertion. This may involve placing a counter electrode at a site adjacent to the skin sites when needles are inserted. An FDA-approved gel may be applied to the skin at the site of the counter electrode before electrode placement. The electrode may have adhesive to stick to the skin. The measurement device is non-invasive and should cause no pain or sensation.

☐ **Examination of skin**

_____ Initial here if you will participate in this procedure.

Your skin will be examined visually by the investigator and/or photographed to determine its color, possible swelling and other changes in appearance. Your skin may also be examined by gentle touching or measuring with a ruler. Your skin may also be imaged using a non-invasive, FDA-approved, ultrasonic scanning device (DermaScan C). It is often used by dermatologists (skin specialists) and the cosmetics industry to image the skin to visualize conditions such as wrinkles, skin aging, scars, and other skin conditions. Skin examination is non-invasive and should cause no pain. For this procedure, each skin site will be examined up to _____ times over a period of up to _____ minutes/hours.

☐ **Skin staining**

_____ Initial here if you will participate in this procedure.

Gentian violet, Betadine (povidone-iodide 10%), Liquid Band-Aid, fluorescein or other FDA-approved treatments for minor skin injuries will be applied to your skin sites. The purpose of this is to label the tiny holes made in the skin by the microneedles or medical needles. The sites will then be examined by the investigator and/or photographed to determine the size, number and distribution

☐ **Transepidermal water loss measurement**

_____ Initial here if you will participate in this procedure.

A device that measures transepidermal water loss (i.e., the rate at which water evaporates from your skin) will be applied to your skin sites. The purpose of this is to

monitor the resealing process of the holes made in your skin. The measurement device is non-invasive and should cause no pain. This is a standard technique commonly used by dermatologists (skin specialists) and in the cosmetics industry. For this procedure, up to _____ measurements will be made at each site over a period of up to _____ hours.

☐ **Electrical resistance measurement**

_____ Initial here if you will participate in this procedure.

A device that measures electrical resistance will be applied to your skin sites. The purpose of this is to monitor the resealing process of the holes made in your skin. This involves placing a measurement electrode on each of your skin sites and placing an additional counter electrode at another adjacent site on the skin. An FDA-approved gel may be applied to the skin at the site of the counter electrode before electrode placement. The electrodes may have adhesive to stick to the skin. The measurement device is non-invasive and should cause no pain. This is an established technique used by dermatologists (skin specialists) and in the cosmetics industry. For this procedure, up to _____ measurements will be made at each site over a period of up to _____ hours.

☐ **Pain assessment**

_____ Initial here if you will participate in this procedure.

You will be asked to assess the sensation and/or pain you experienced during one or more of the procedures. You may be asked to rate the pain using a sliding-scale device. You may also be asked to describe it verbally. You may be asked to provide additional information about your observations and feelings about one or more of the procedures.

☐ **Survey**

_____ Initial here if you will participate in this procedure.

You will be asked to complete a survey using a computer. The survey includes questions about any pain you felt and your preferences for different procedures that you experience in this study. You will be also asked about your experience with flu vaccines and your personal characteristics, such as your age and gender. The investigator will ask you about how we can improve the devices in this study. Your answer to that question will be recorded using a digital recorder. One password-protected copy of the recording will be stored on a Georgia Tech computer and be accessible to study personnel only. The purpose of the recording is to gather feedback about how to improve devices used in this study.

COMPENSATION: You will receive $____ for your participation in this study. If you withdraw early after you start the study procedure, you will receive partial compensation of 50%, which will be given to you at the time of withdrawal. You will not receive any compensation for the prescreening procedure. For procedures that require more than six hours of continuous participation, you will receive free meals.

RISKS: Every study involves some risk. There is a small chance that an infection may occur at the skin site. However, we will take precautions to prevent infections, such as using sterilized materials and wiping the skin with an alcohol wipe before the study. Pressing the microneedles

into the skin is expected to cause little, if any, pain. Use of a hypodermic needle may cause pain. There is a chance that you may get a small blemish, scratch, or bruise at the site of application of the needles. When applying suction to the skin, there may be mild pain and there is a small chance of forming a blister in the skin.

There is also an extremely small chance of the microneedles breaking and staying in the layers of the skin. If pieces of a microneedle break off in the outer layer (i.e., epidermis) of your skin, they should fall off through normal skin renewal and are unlikely to have adverse effects. If they are embedded deeper in your skin, there is a very small chance that a foreign-body granuloma (a growth of cells around the microneedle pieces) may occur. This is an unpleasant condition, but not life threatening. The granuloma may take up to several months to appear, and can be removed by a physician. However, the possibility of granuloma formation is expected to be extremely small. If you develop a granuloma, you are requested to notify the Contact Persons listed at the end of this form.

The microneedles may be made from metal, plastic, and glass. Metals to be used include stainless steel, titanium, gold and other metals known to be safe for use in the body. Nickel may also be used; see below for associated risks. Plastics to be used include polymethylmethacrylate, polylactic acid, polyglycolic acid, polylactic-co-glycolic acid and other polymers known to be safe for use in the body. Glass, including borosilicate glass, may be used, which is known to be safe for use in the body.

Due to the experimental nature of this study there may be other risks that are currently unknown. If during this study, significant new findings become available that may affect your willingness to continue participation in this study, you will be informed of the findings.

For studies that involve exposure to nickel. There is some chance that exposure to nickel will result in a local reaction, with redness, possible itching and a watery blister (e.g., like a mosquito bite). Such a reaction usually clears up once contact with nickel is stopped.

BENEFITS: There are no direct benefits of this research to you as a participant. However, there may be a benefit to society from the understanding gained from your participation. If successful, this project could enable painless administration of drugs that normally require hypodermic shots and physiological measurements that normally require drawing blood

CONFIDENTIALITY: All information concerning you will be kept private. Research records from this study may be obtained by court order. If information about you is published, it will be written in a way that you cannot be recognized.

By signing this form, you are giving permission for the organizers of this study to allow additional researchers who are collaborating with the organizers, as well as government (the Food and Drug Administration) and university regulatory agencies, to review the information regarding your participation in the study. All data and medical records associated with your participation in this study will be kept confidential except where noted, and as may be required by law. You will be identified by your initials or a code number and not your name whenever possible, including to the collaborators and regulatory agency.

IN CASE OF INJURY OR HARM: If you are injured as a result of being in this study, please contact Principal Investigator, Mark Prausnitz, Ph.D., at telephone (404) 894-5135. Neither the Principal Investigator nor Georgia Institute of Technology has made provision for payment of

costs associated with any injury resulting from participation in this study. If sufficiently urgent, 911 will be called.

COSTS TO YOU: There is no cost to you to participate in this study. In the unlikely event that you are injured or harmed through participation in this study, it is your responsibility to arrange for payments of any medical treatment cost from your insurance provider or other source.

ALTERNATIVES: For any medical treatments, there are usually alternative treatments to consider. In this study the only alternative is the option not to participate in this study.

VOLUNTARY PARTICIPATION/WITHDRAWAL: Participation in this study is voluntary. You are free to withdraw from participation at any time. Your decision to participate or not participate will not negatively affect employment or (if you are a student) student status, grades, or class credit.

CONFLICT OF INTEREST: The Principal Investigator and the Georgia Institute of Technology have a financial interest in this study.

CONTACT PERSONS: To ask questions about this study or to report research related injuries, contact Mark Prausnitz, Ph.D., at (404) 894-5135.

If you have any questions or concerns about your rights as a participant in this research study, contact the Compliance Administrator of the Office of Research Compliance, Georgia Institute of Technology at (404) 894-6942.

A copy of this consent form will be given to you.

Your signature below indicates that you consent to volunteer for this study.

_____	_____	
Subject name (PRINTED)	Subject code #	
_____	_____	_____
Subject signature	Date	Time
_____	_____	_____
Investigator signature	Date	Time

B.5 Checklist for Self-Vaccination Study

H08338 Pre-Flight Checklist

Materials

Safety

- ☐AED
- ☐First Aid Supplies
- ☐Keys
- ☐CPR Instructions
- ☐Charged phone w/ service and pre-programmed #s
  - Dr. P Cell Phone: [redacted]
  - Dr. F Cell Phone:
  - GT Police: 404-894-2500
  - Concentra: 404-881-1155
- ☐Phone charger
- ☐Gloves

IM Injection Materials

- ☐Safety needles + syringes(200, 260 lb cutoff)
- ☐Antibiotic-free saline (2 vials, 1 for diluents)
- ☐10+ alcohol wipes for vial and for skin
- ☐Sharps container
- ☐Latex-free Band-Aids

Microneedle Injection Materials

- ☐Purell
- ☐Gauze Pads
- ☐4 microneedle devices with labels and ETO indications (backups ready)
- ☐Laminated instructions
- ☐Fluorescein and gentian violet
- ☐(1) 3 mL syringe, (1) 1 mL syringe, (2) needles: for diluting dye
- ☐Sterile swabs (4) + sterile specimen container (1) for applying dye
- ☐Camera, charger, and blue lighting

Other Study Materials

- ☐Phone numbers and names for day's worth of volunteers
- ☐Money, sign-in, 2 signature receipts, receipt book
- ☐Laptop with Survey Program + Audacity
- ☐Computer microphone (backup of Coby Recorder)
- ☐Data sheet for microneedle pokes
- ☐Small trash can and trash bag for saline, gloves, etc.
- ☐Lab coat
- ☐Consent form, 2 pens, 2 highlighters

B.6 Data Sheet for Self-Vaccination Study

Data Sheet

Volunteer ID: _____

Order of Procedures: 1._____ 2._____ 3._____

Height:

Weight:

Favorite method of administration (SA home, etc):

Self-Administration 1:

Estimate number of missing dots out of fifty: _____

Picture taken: Y / N Picture number: _____

Bleeding: Y / N Needles in skin: Y / N

Self-Administration 2:

Estimate number of missing dots out of fifty: _____

Picture taken: Y / N Picture number: _____

Bleeding: Y / N Needles in skin: Y / N

Self-Administration 3:

Estimate number of missing dots out of fifty: _____

Picture taken: Y / N Picture number: _____

Bleeding: Y / N Needles in skin: Y / N

Investigator-Administration:

Estimate number of missing dots out of fifty: _____

Picture taken: Y / N Picture number: _____

Bleeding: Y / N Needles in skin: Y / N

Intramuscular Injection:

Bleeding: Y / N

Notes and Observations:

REFERENCES

1. Hanas, R. and J. Ludvigsson, *Experience of pain from insulin injections and needle-phobia in young patients with IDDM.* Practical Diabetes International, 1997. **14**(4): p. 95-99.

2. Howe, C.J., et al., *Needle anxiety in children with type 1 diabetes and their mothers.* MCN Am J Matern Child Nurs, 2011. **36**(1): p. 25-31.

3. Simmons, J.H., et al., *Reliability of the Diabetes Fear of Injecting and Self-Testing Questionnaire in pediatric patients with type 1 diabetes.* Diabetes Care, 2007. **30**(4): p. 987-8.

4. Renard, E., et al., *Closed-loop insulin delivery using a subcutaneous glucose sensor and intraperitoneal insulin delivery: feasibility study testing a new model for the artificial pancreas.* Diabetes Care, 2010. **33**(1): p. 121-7.

5. Kennedy, E.D., et al., *March Flu Vaccination Coverage*, 2012, Centers for Disease Control and Prevention.

6. Thompson, W.W., et al., *Influenza-associated hospitalizations in the United States.* JAMA, 2004. **292**(11): p. 1333-40.

7. Prosser, L.A., et al., *Non-traditional settings for influenza vaccination of adults: costs and cost effectiveness.* Pharmacoeconomics, 2008. **26**(2): p. 163-78.

8. Kim, Y.C., J.H. Park, and M.R. Prausnitz, *Microneedles for drug and vaccine delivery.* Adv Drug Deliv Rev, 2012.

9. Pettis, R.J. and A.J. Harvey, *Microneedle Delivery: Clinical Studies and Emerging Medical Applications.* Therapeutic Delivery, 2012. **3**(3): p. 357-371.

10. Gupta, J., E.I. Felner, and M.R. Prausnitz, *Rapid pharmacokinetics of intradermal insulin administered using microneedles in type 1 diabetes subjects.* Diabetes Technol Ther, 2011. **13**(4): p. 451-6.

11. Pettis, R.J., et al., *Intradermal microneedle delivery of insulin lispro achieves faster insulin absorption and insulin action than subcutaneous injection.* Diabetes Technol Ther, 2011. **13**(4): p. 435-42.

12. Cryz, S.J., Jr., *Patient compliance in the use of Vivotif Berna(R) vaccine, typhoid vaccine, live oral Ty21a.* J Travel Med, 1998. **5**(1): p. 14-7.

13. Warren, T.A., et al., *A cost-effectiveness analysis of typhoid fever vaccines in US military personnel.* Pharmacoeconomics, 1996. **10**(5): p. 475-83.

14. Ochiai, R.L., et al., *The use of typhoid vaccines in Asia: the DOMI experience.* Clin Infect Dis, 2007. **45 Suppl 1**: p. S34-8.

15. Coleman, B.L., et al., *A randomized control trial comparing immunogenicity, safety, and preference for self- versus nurse-administered intradermal influenza vaccine.* Vaccine, 2012. **30**(44): p. 6287-93.

16. Rader, R.A., *(Re)defining biopharmaceutical.* Nat Biotechnol, 2008. **26**(7): p. 743-51.

17. Singh, R., S. Singh, and J.W. Lillard, Jr., *Past, present, and future technologies for oral delivery of therapeutic proteins.* J Pharm Sci, 2008. **97**(7): p. 2497-523.

18. Brown, L.R., *Commercial challenges of protein drug delivery.* Expert Opin Drug Deliv, 2005. **2**(1): p. 29-42.

19. Tucker, S.N., D.W. Tingley, and C.D. Scallan, *Oral adenoviral-based vaccines: historical perspective and future opportunity.* Expert Rev Vaccines, 2008. **7**(1): p. 25-31.

20. Prausnitz, M.R., S. Mitragotri, and R. Langer, *Current status and future potential of transdermal drug delivery.* Nat Rev Drug Discov, 2004. **3**(2): p. 115-24.

21. Prausnitz, M.R. and R. Langer, *Transdermal drug delivery.* Nat Biotechnol, 2008. **26**(11): p. 1261-8.

22. Choy, Y.B. and M.R. Prausnitz, *The rule of five for non-oral routes of drug delivery: ophthalmic, inhalation and transdermal.* Pharm Res, 2011. **28**(5): p. 943-8.

23. Nwe, N., et al., *Expression of hemagglutinin protein from the avian influenza virus H5N1 in a baculovirus/insect cell system significantly enhanced by suspension culture.* BMC Microbiol, 2006. **6**: p. 16.

24. American Diabetes, A., *Insulin administration.* Diabetes Care, 2004. **27 Suppl 1**: p. S106-9.

25. National Center for Immunization and Respiratory Diseases, *General recommendations on immunization --- recommendations of the Advisory Committee on Immunization Practices (ACIP).* MMWR Recomm Rep, 2011. **60**(2): p. 1-64.

26. Zehrung, D. and D. Kristensen, *Intradermal Delivery of Vaccines*, 2009, PATH.

27. Tarnow, K. and N. King, *Intradermal injections: traditional bevel up versus bevel down.* Appl. Nurs. Res., 2004. **17**(4): p. 275-82.

28. Mollema, E.D., et al., *Phobia of self-injecting and self-testing in insulin-treated diabetes patients: opportunities for screening.* Diabet Med, 2001. **18**(8): p. 671-4.

29. Kim, Y.C., et al., *Delivery systems for intradermal vaccination.* Curr Top Microbiol Immunol, 2012. **351**: p. 77-112.

30. Mota, B.E., et al., *Adverse events post smallpox-vaccination: insights from tail scarification infection in mice with Vaccinia virus.* PLoS One, 2011. **6**(4): p. e18924.

31. Mitragotri, S., *Current status and future prospects of needle-free liquid jet injectors.* Nat Rev Drug Discov, 2006. **5**(7): p. 543-8.

32. Kuo, C.F., J.H. Wang, and N.S. Yang, *Direct gene transfer and vaccination via skin transfection using a gene gun.* Methods Mol Med, 2002. **69**: p. 137-47.

33. Pokorna, D., et al., *Vaccination with human papillomavirus type 16-derived peptides using a tattoo device.* Vaccine, 2009. **27**(27): p. 3519-29.

34. Prausnitz, M.R., *Microneedles for transdermal drug delivery.* Adv Drug Deliv Rev, 2004. **56**(5): p. 581-7.

35. Wermeling, D.P., et al., *Microneedles permit transdermal delivery of a skin-impermeant medication to humans.* Proc Natl Acad Sci U S A, 2008. **105**(6): p. 2058-63.

36. Andrews, S., et al., *Transdermal insulin delivery using microdermabrasion.* Pharm Res, 2011. **28**(9): p. 2110-8.

37. Polat, B.E., et al., *Ultrasound-mediated transdermal drug delivery: mechanisms, scope, and emerging trends.* J Control Release, 2011. **152**(3): p. 330-48.

38. Banga, A.K. and M.R. Prausnitz, *Assessing the potential of skin electroporation for the delivery of protein- and gene-based drugs.* Trends Biotechnol, 1998. **16**(10): p. 408-12.

39. Kim, Y.C., P.J. Ludovice, and M.R. Prausnitz, *Transdermal delivery enhanced by magainin pore-forming peptide.* J Control Release, 2007. **122**(3): p. 375-83.

40. Lee, J.W., et al., *Microsecond thermal ablation of skin for transdermal drug delivery.* J Control Release, 2011. **154**(1): p. 58-68.

41. Gupta, J., E.I. Felner, and M.R. Prausnitz, *Minimally invasive insulin delivery in subjects with type 1 diabetes using hollow microneedles.* Diabetes Technol Ther, 2009. **11**(6): p. 329-37.

42. Pettis, R.J., et al., *Microneedle-based intradermal versus subcutaneous administration of regular human insulin or insulin lispro: pharmacokinetics and postprandial glycemic excursions in patients with type 1 diabetes.* Diabetes Technol Ther, 2011. **13**(4): p. 443-50.

43. Daddona, P.E., et al., *Parathyroid hormone (1-34)-coated microneedle patch system: clinical pharmacokinetics and pharmacodynamics for treatment of osteoporosis.* Pharm Res, 2011. **28**(1): p. 159-65.

44. Harvey, A.J., et al., *Microneedle-based intradermal delivery enables rapid lymphatic uptake and distribution of protein drugs.* Pharm Res, 2011. **28**(1): p. 107-16.

45. Bollinger, A., *Microlymphatics of human skin.* Int J Microcirc Clin Exp, 1993. **12**(1): p. 1-15.

46. Kretsos, K. and G.B. Kasting, *Dermal capillary clearance: physiology and modeling.* Skin Pharmacol Physiol, 2005. **18**(2): p. 55-74.

47. Glenn, G.M. and R.T. Kenney, *Mass vaccination: solutions in the skin.* Curr Top Microbiol Immunol, 2006. **304**: p. 247-68.

48. Huang, C.M., *Topical vaccination: the skin as a unique portal to adaptive immune responses.* Semin Immunopathol, 2007. **29**(1): p. 71-80.

49. Kim, Y.C., et al., *Enhanced memory responses to seasonal H1N1 influenza vaccination of the skin with the use of vaccine-coated microneedles.* J Infect Dis, 2010. **201**(2): p. 190-8.

50. Quan, F.S., et al., *Intradermal vaccination with influenza virus-like particles by using microneedles induces protection superior to that with intramuscular immunization.* J Virol, 2010. **84**(15): p. 7760-9.

51. Ansaldi, F., et al., *Intanza((R)) 15mcg intradermal influenza vaccine elicits cross-reactive antibody responses against heterologous A(H3N2) influenza viruses.* Vaccine, 2012. **30**(18): p. 2908-13.

52. Fernando, G.J., et al., *Nanopatch targeted delivery of both antigen and adjuvant to skin synergistically drives enhanced antibody responses.* J Control Release, 2012. **159**(2): p. 215-21.

53. Quan, F.S., et al., *Dose sparing enabled by skin immunization with influenza virus-like particle vaccine using microneedles.* J Control Release, 2010. **147**(3): p. 326-32.

54. van Damme, P., et al., *Safety and efficacy of a novel microneedle device for dose sparing intradermal influenza vaccination in healthy adults.* Vaccine, 2009. **27**(3): p. 454-9.

55. Murphy, K., et al., *Janeway's immunobiology*. 8th ed. 2012, New York: Garland Science. xix, 868 p.

56. Hirsch, L.J., et al., *Comparative glycemic control, safety and patient ratings for a new 4 mm x 32G insulin pen needle in adults with diabetes.* Curr Med Res Opin, 2010. **26**(6): p. 1531-41.

57. Eizenberg, P., et al., *Acceptance of Intanza(R) 9 mug intradermal influenza vaccine in routine clinical practice in Australia and Argentina.* Adv Ther, 2011. **28**(8): p. 640-9.

58. Raphael, A.P., et al., *Targeted, needle-free vaccinations in skin using multilayered, densely-packed dissolving microprojection arrays.* Small, 2010. **6**(16): p. 1785-93.

59. Henry, S., et al., *Microfabricated microneedles: A novel approach to transdermal drug delivery.* J Pharm Sci, 1999. **88**(9): p. 948.

60. Patel, S.R., et al., *Suprachoroidal drug delivery to the back of the eye using hollow microneedles.* Pharm Res, 2011. **28**(1): p. 166-76.

61. Han, S.W., et al., *High-efficiency DNA injection into a single human mesenchymal stem cell using a nanoneedle and atomic force microscopy.* Nanomedicine, 2008. **4**(3): p. 215-25.

62. Gerstel, M.S. and V.A. Place, *Drug delivery device*, 1976, Alza Corporation: United States Patent.

63. Henry, S., et al., *Microfabricated microneedles: a novel approach to transdermal drug delivery.* J Pharm Sci, 1998. **87**(8): p. 922-5.

64. Prausnitz, M.R., et al., *Microneedle-based vaccines.* Curr Top Microbiol Immunol, 2009. **333**: p. 369-93.

65. Gonzalez-Gonzalez, E., et al., *Silencing of reporter gene expression in skin using siRNAs and expression of plasmid DNA delivered by a soluble protrusion array device (PAD).* Mol Ther, 2010. **18**(9): p. 1667-74.

66. Neutra, M.R. and P.A. Kozlowski, *Mucosal vaccines: the promise and the challenge.* Nat Rev Immunol, 2006. **6**(2): p. 148-58.

67. Gupta, J., et al., *Infusion pressure and pain during microneedle injection into skin of human subjects.* Biomaterials, 2011. **32**(28): p. 6823-31.

68. Lee, J.W., J.H. Park, and M.R. Prausnitz, *Dissolving microneedles for transdermal drug delivery.* Biomaterials, 2008. **29**(13): p. 2113-24.

69. Roxhed, N., et al., *Penetration-enhanced ultrasharp microneedles and prediction on skin interaction for efficient transdermal drug delivery.* Journal of Microelectromechanical Systems, 2007. **16**(6): p. 1429-1440.

70. Davis, S.P., et al., *Insertion of microneedles into skin: measurement and prediction of insertion force and needle fracture force.* J Biomech, 2004. **37**(8): p. 1155-63.

71. Gill, H.S. and M.R. Prausnitz, *Coating formulations for microneedles.* Pharm Res, 2007. **24**(7): p. 1369-80.

72. Chu, L.Y., S.O. Choi, and M.R. Prausnitz, *Fabrication of dissolving polymer microneedles for controlled drug encapsulation and delivery: Bubble and pedestal microneedle designs.* J Pharm Sci, 2010. **99**(10): p. 4228-38.

73. Park, J.H. and M.R. Prausnitz, *Analysis of Mechanical Failure of Polymer Microneedles by Axial Force.* J Korean Phys Soc, 2010. **56**(4): p. 1223-1227.

74. Donnelly, R.F., et al., *Microneedle-mediated transdermal and intradermal drug delivery*. 2012, Chichester, West Sussex: Wiley-Blackwell.

75. Gill, H.S. and M.R. Prausnitz, *Coating Formulations for Microneedles.* Pharmaceutical Research, 2007. **24**(7).

76. McGrath, M.G., et al., *Determination of parameters for successful spray coating of silicon microneedle arrays.* Int J Pharm, 2011. **415**(1-2): p. 140-9.

77. DeMuth, P.C., et al., *Nano-layered microneedles for transcutaneous delivery of polymer nanoparticles and plasmid DNA.* Adv Mater, 2010. **22**(43): p. 4851-6.

78. Kim, Y.C., et al., *Formulation and coating of microneedles with inactivated influenza virus to improve vaccine stability and immunogenicity.* J Control Release, 2010. **142**(2): p. 187-95.

79. Chen, X., et al., *Improving the reach of vaccines to low-resource regions, with a needle-free vaccine delivery device and long-term thermostabilization.* J Control Release, 2011. **152**(3): p. 349-55.

80. Sullivan, S.P., N. Murthy, and M.R. Prausnitz, *Minimally invasive protein delivery with rapidly dissolving polymer microneedles.* Adv. Mater., 2008. **20**: p. 933-938.

81. Chu, L.Y., *Dissolving microneedles for cutaneous drug and vaccine delivery*, in *Biomedical Engineering*2009, Georgia Institute of Technology. p. 151.

82. Chu, L.Y. and M.R. Prausnitz, *Separable arrowhead microneedles.* J Control Release, 2011. **149**(3): p. 242-9.

83. Davis, S.P., et al., *Hollow metal microneedles for insulin delivery to diabetic rats.* IEEE Trans Biomed Eng, 2005. **52**(5): p. 909-15.

84. McPhee, S., M.A. Papadakis, and M.W. Rabow, *Current Medical Diagnosis and Treatment*. 2012: McGraw Hill.

85. Centers for Disease Control and Prevention, *National diabetes fact sheet: national estimates and general information on diabetes and prediabetes in the United States, 2011*, U.S. Department of Health and Human Services, Editor 2011: Atlanta, GA.

86. Pampanelli, S., et al., *Improved postprandial metabolic control after subcutaneous injection of a short-acting insulin analog in IDDM of short duration with residual pancreatic beta-cell function.* Diabetes Care, 1995. **18**(11): p. 1452-9.

87. Steiner, S., et al., *A novel insulin formulation with a more rapid onset of action.* Diabetologia, 2008. **51**(9): p. 1602-6.

88. Renard, E., *Insulin delivery route for the artificial pancreas: subcutaneous, intraperitoneal, or intravenous? Pros and cons.* J Diabetes Sci Technol, 2008. **2**(4): p. 735-8.

89. Mack, G.S., *Pfizer dumps Exubera.* Nat Biotechnol, 2007. **25**(12): p. 1331-2.

90. U.S. Food and Drug Adminsitration. *Complete List of Vaccines Licensed for Immunization and Distribution in the US*. 2012 [cited 2012 06/18/12]; Available from: http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM093833.

91. Flach, T.L., et al., *Alum interaction with dendritic cell membrane lipids is essential for its adjuvanticity.* Nat Med, 2011. **17**(4): p. 479-87.

92. Plotkin, S.A. and W.A. Orenstein, *Vaccines*. 5th ed. 2008, Philadelphia, Pa.: Saunders. 1725 p.

93. Amorij, J.P., et al., *Development of stable influenza vaccine powder formulations: challenges and possibilities.* Pharm Res, 2008. **25**(6): p. 1256-73.

94. MedImmune, *Media Backgrounder: About FluMist*, 2010: http://pressroom.medimmune.com/wp-content/uploads/2011/06/Media-Backgrounder-About-FluMist-2010.pdf.

95. Coelingh, K. *FluMist Update*. in *National Influenza Vaccine Summit*. 2010. Phoenix, AZ.

96. Centers for Disease Control and Prevention. *Vaccine Information Statement: Inactivated Influenza Vaccine*. 2011 06/19/12]; Available from: http://www.cdc.gov/vaccines/pubs/vis/downloads/vis-flu.pdf.

97. Osterholm, M.T., et al., *Efficacy and effectiveness of influenza vaccines: a systematic review and meta-analysis.* Lancet Infect Dis, 2012. **12**(1): p. 36-44.

98. Centers for Disease Control and Prevention. *Flu Vaccine Effectiveness: Questions and Answers for Healthcare Professionals*. 2011 06/19/2012]; Available from: http://www.cdc.gov/flu/professionals/vaccination/effectivenessqa.htm.

99. Centers for Disease Control and Prevention. *Fluzone High-Dose Seasonal Influenza Vaccine: Questions and Answers*. 2011 06/19/2012]; Available from: http://www.cdc.gov/flu/protect/vaccine/qa_fluzone.htm.

100. Young, F. and F. Marra, *A systematic review of intradermal influenza vaccines.* Vaccine, 2011. **29**(48): p. 8788-801.

101. American Pharmacists Association, *Pharmacy-Based Immunization Delivery*. 2009, Washington, D.C.: American Pharmacists Association. 235.

102. World Health Organization. *WHO Prequalified Vaccines*. 2011 06/21/12]; Available from: http://www.who.int/immunization_standards/vaccine_quality/PQ_vaccine_list_en/en/index.html.

103. Mitragotri, S., *Immunization without needles.* Nat Rev Immunol, 2005. **5**(12): p. 905-16.

104. Giudice, E.L. and J.D. Campbell, *Needle-free vaccine delivery.* Adv Drug Deliv Rev, 2006. **58**(1): p. 68-89.

105. Garrison, L.P., Jr., et al., *Using cost-effectiveness analysis to support research and development portfolio prioritization for product innovations in measles vaccination.* J Infect Dis, 2011. **204 Suppl 1**: p. S124-32.

106. Amorij, J.P., et al., *Needle-free influenza vaccination.* Lancet Infect Dis, 2010. **10**(10): p. 699-711.

107. American Pharmacists Association. *Nation's Pharmacists Ready to Administer Seasonal Flu Vaccines*. 2011 06/21/12]; Available from: Nation's Pharmacists Ready to Administer Seasonal Flu Vaccines.

108. U.S. Agency for International Development, *Immunization Essentials: A Practical Field Guide*. 2003.

109. Ajzen, I. and M. Fishbein, *Understanding attitudes and predicting social behavior*. 1980, Englewood Cliffs, N.J.: Prentice-Hall;. x, 278 p.

110. Sheppard, B.H., J. Hartwick, and P.R. Warshaw, *The Theory of Reasoned Action - a Meta-Analysis of Past Research with Recommendations for Modifications and Future-Research.* Journal of Consumer Research, 1988. **15**(3): p. 325-343.

111. Ajzen, I., *The theory of planned behaviour: Reactions and reflections.* Psychology & Health, 2011. **26**(9): p. 1113-1127.

112. Janz, N.K. and M.H. Becker, *The Health Belief Model: a decade later.* Health Educ Q, 1984. **11**(1): p. 1-47.

113. Salant, P. and D.A. Dillman, *How to Conduct Your Own Survey*. 1994.

114. Gorsuch, R.L., *Factor analysis*. 2nd ed. 1983, Hillsdale, N.J.: L. Erlbaum Associates. xvii, 425 p.

115. Frew, P.M., et al., *An extended model of reasoned action to understand the influence of individual- and network-level factors on African Americans' participation in HIV vaccine research.* Prev Sci, 2010. **11**(2): p. 207-18.

116. Myers, L.B. and R. Goodwin, *Determinants of adults' intention to vaccinate against pandemic swine flu.* BMC Public Health, 2011. **11**(1): p. 15.

117. Painter, J.E., et al., *Psychosocial correlates of intention to receive an influenza vaccination among rural adolescents.* Health Educ Res, 2010. **25**(5): p. 853-64.

118. Godin, G., L.A. Vezina-Im, and H. Naccache, *Determinants of influenza vaccination among healthcare workers.* Infect Control Hosp Epidemiol, 2010. **31**(7): p. 689-93.

119. Painter, J.E., et al., *Adolescent attitudes toward influenza vaccination and vaccine uptake in a school-based influenza vaccination intervention: a mediation analysis.* J Sch Health, 2011. **81**(6): p. 304-12.

120. Ben Natan, M., et al., *Attitude of Israeli mothers with vaccination of their daughters against human papilloma virus.* J Pediatr Nurs, 2011. **26**(1): p. 70-7.

121. Reynolds, D. and K.A. O'Connell, *Testing a Model for Parental Acceptance of Human Papillomavirus Vaccine in 9- to 18-Year-Old Girls: A Theory-Guided Study.* J Pediatr Nurs, 2011.

122. Dillard, J.P., *An application of the integrative model to women's intention to be vaccinated against HPV: implications for message design.* Health Communication, 2011. **26**(5): p. 479-86.

123. Manhart, L.E., et al., *HPV vaccination among a community sample of young adult women.* Vaccine, 2011. **29**(32): p. 5238-44.

124. Marlow, L.A., J. Waller, and J. Wardle, *Parental attitudes to pre-pubertal HPV vaccination.* Vaccine, 2007. **25**(11): p. 1945-52.

125. Zimmerman, R.K., et al., *Predictors of pneumococcal polysaccharide vaccination among patients at three inner-city neighborhood health centers.* Am J Geriatr Pharmacother, 2005. **3**(3): p. 149-59.

126. Dube, E., et al., *New vaccines offering a larger spectrum of protection against acute otitis media: will parents be willing to have their children immunized?* Int J Pediatr Otorhinolaryngol, 2009. **73**(7): p. 987-91.

127. Rhodes, S.D. and R.J. Diclemente, *Psychosocial predictors of hepatitis B vaccination among young African-American gay men in the deep south.* Sexually Transmitted Diseases, 2003. **30**(5): p. 449-54.

128. Bonney, L.E., et al., *Correlates of acceptance of a hypothetical Gonorrhea vaccine by incarcerated women.* Sexually Transmitted Diseases, 2007. **34**(10): p. 778-782.

129. Gagnon, M.P. and G. Godin, *Young adults and HIV vaccine: determinants of the intention of getting immunized.* Can J Public Health, 2000. **91**(6): p. 432-4.

130. Liau, A. and G.D. Zimet, *Undergraduates' perception of HIV immunization: attitudes and behaviours as determining factors.* Int J STD AIDS, 2000. **11**(7): p. 445-50.

131. Miller, M.A. and A.R. Hinman, *Economic analyses of vaccine policies*, in *Vaccines*, S.A. Plotkin, W.A. Orenstein, and P.A. Offit, Editors. 2008, Saunders.

132. Lee, B.Y. and S.M. McGlone, *Pricing of new vaccines.* Hum Vaccin, 2010. **6**(8): p. 619-26.

133. Meltzer, M.I., *Introduction to health economics for physicians.* Lancet, 2001. **358**(9286): p. 993-8.

134. Lieu, T., M.I. Meltzer, and M.L. Messonier, *Guidance for Health Economics Studies Presented to the Advisory Committee on Immunization Practices (ACIP)*, CDC, Editor 2007: Atlanta.

135. Nord, E., *Cost-value analysis in health care : making sense out of QALYs*. Cambridge studies in philosophy and public policy. 1999, Cambridge England ; New York: Cambridge University Press. xxiii, 157 p.

136. McGregor, M., *Cost-utility analysis: Use QALYs only with great caution.* Canadian Medical Association Journal, 2003. **168**(4): p. 433-434.

137. Edmunds, W.J., G.F. Medley, and D.J. Nokes, *Evaluating the cost-effectiveness of vaccination programmes: A dynamic perspective.* Statistics in medicine, 1999. **18**(23): p. 3263-3282.

138. Ekwueme, D.U., B.G. Weniger, and R.T. Chen, *Model-based estimates of risks of disease transmission and economic costs of seven injection devices in sub-Saharan Africa.* Bull World Health Organ, 2002. **80**(11): p. 859-70.

139. Pereira, C.C. and D. Bishai, *Vaccine presentation in the USA: economics of prefilled syringes versus multidose vials for influenza vaccination.* Expert Rev Vaccines, 2010. **9**(11): p. 1343-1349.

140. Hickling, J., et al., *Improving the Affordability of Inactivated Poliovirus Vaccines (IPV) for Use in Low- and Middle-Income Countries: An Economic Analysis of Strategies to Reduce the Cost of Routine IPV Immunization*, 2010, PATH.

141. Griffiths, U.K., et al., *Incremental costs of introducing jet injection technology for delivery of routine childhood vaccinations: Comparative analysis from Brazil, India, and South Africa.* Vaccine, 2011. **29**(5): p. 969-975.

142. Levin, C.E., et al., *The costs of home delivery of a birth dose of hepatitis B vaccine in a prefilled syringe in Indonesia.* Bull World Health Organ, 2005. **83**(6): p. 456-61.

143. Hibbert, C.L., et al., *Cost-effectiveness of live-attenuated influenza vaccine, trivalent in preventing influenza in young children attending day-care centres.* Vaccine, 2007. **25**(47): p. 8010-20.

144. You, S.K., et al., *Effect of applying modes of the polymer microneedle-roller on the permeation of L-ascorbic acid in rats.* J Drug Target, 2010. **18**(1): p. 15-20.

145. Walker, H.K., W.D. Hall, and J.W. Hurst, *Clinical methods : the history, physical, and laboratory examinations*. 3rd ed. 1990, Boston: Butterworths. xxxi, 1087 p.

146. Li, C.G., et al., *An optimized hollow microneedle for minimally invasive blood extraction.* Biomed. Microdevices, 2012.

147. Perennes, F., et al., *Sharp beveled tip hollow microneedle arrays fabricated by LIGA and 3D soft lithography with polyvinyl alcohol.* Journal of Micromechanics and Microengineering, 2006. **16**: p. 473-479.

148. Stoeber, B. and D. Liepmann, *Arrays of hollow out-of-plane microneedles for drug delivery.* Journal of Microelectromechanical Systems, 2005. **14**(3): p. 472-479.

149. S.J., M. and L. S.S., *A novel fabrication method of a microneedle array using inclined deep x-ray exposure.* Journal of Micromechanics and Microengineering, 2005. **15**: p. 903-911.

150. J.M., L., G. E.J., and P. A.P., *Polymer investment molding: method for fabricating hollow, microscale parts.* Sensors and Actuators A: Physical, 2007. **134**: p. 2-10.

151. A., O., *Two Photon Polymerization of Polymer-Ceramic Hybrid Materials for Transdermal Drug Delivery.* Int. J. Appl. Ceram. Tech., 2007. **4**(1): p. 22-29.

152. Alarcon, J.B., et al., *Preclinical evaluation of microneedle technology for intradermal delivery of influenza vaccines.* Clin Vaccine Immunol, 2007. **14**(4): p. 375-81.

153. Burton, S.A., et al., *Rapid intradermal delivery of liquid formulations using a hollow microstructured array.* Pharm Res, 2011. **28**(1): p. 31-40.

154. K., K., et al., *A tapered hollow metallic microneedle array using backside exposure of SU-8.* Journal of Micromechanics and Microengineering, 2004. **14**: p. 597-603.

155. Laurent, P.E., et al., *Evaluation of the clinical performance of a new intradermal vaccine administration technique and associated delivery system.* Vaccine, 2007. **25**(52): p. 8833-42.

156. Gill, H.S., et al., *Effect of microneedle design on pain in human volunteers.* Clin J Pain, 2008. **24**(7): p. 585-94.

157. Birchall, J.C., et al., *Microneedles in clinical practice--an exploratory study into the opinions of healthcare professionals and the public.* Pharm Res, 2011. **28**(1): p. 95-106.

158. Davis, S.P., et al., *Insertion of microneedles into skin: measurement and prediction of insertion force and needle fracture force.* J. Biomech., 2004. **37**(8): p. 1155-63.

159. Griss, P. and G. Stemme, *Side-Opened Out-of-Plane Microneedles for microfluidic trasdermal liquid transfer.* Journal of Microelectromechanical Systems, 2003. **12**(3): p. 296-301.

160. Martanto, W., et al., *Mechanism of fluid influsion during microneedle insertion and retraction.* Journal of Controlled Release, 2006. **112**: p. 357-361.

161. Davis, S.P., W. Martanto, and M.G. Allen, *Hollow Metal Microneedles for Insulin Delivery to Diabetic Rats.* IEEE Transactions on Biomedical Engineering, 2005. **52**(5): p. 909-914.

162. Matteucci, M., et al., *Poly vinyl alcohol re-usable masters for microneedle replication.* Microelectronic Engineering, 2009.

163. Xu, Y., et al., *Methods and moulds for use in fabricating side-ported microneedles*, W.I.P. Organization, Editor 2005.

164. Kim, H. and J.S. Colton, *Fabrication and analysis of plastic hypodermic needles.* J Med Eng Technol, 2005. **29**(4): p. 181-6.

165. Zhang, M., et al., *Fabrication of ZnO nanowire devices via selective electrodeposition.* Electrochemical and Solid State Letters, 2008. **11**(9): p. D69-D71.

166. Park, J.H., M.G. Allen, and M.R. Prausnitz, *Biodegradable polymer microneedles: fabrication, mechanics and transdermal drug delivery.* J Control Release, 2005. **104**(1): p. 51-66.

167. Ploin, D., et al., *Echographic measurement of skin thickness in sites suitable for intradermal vaccine injection in infants and children.* Vaccine, 2011. **29**(46): p. 8438-42.

168. Stoeber, B. and D. Liepmann, *Arrays of Hollow Out-of-Plane Microneedles for Drug Delivery.* Journal of Microelectromechanical Systems, 2005. **14**(3): p. 472.

169. Lee, K., et al., *Drawing lithography: three-dimensional fabrication of an ultrahigh-aspect-ratio microneedle.* Adv Mater, 2010. **22**(4): p. 483-6.

170. Rzeszut, J.R., *Children with diabetes: the impact of fear of needles.* J Pediatr Nurs, 2011. **26**(6): p. 589-92.

171. Hanas, R., *Reducing injection pain in children and adolescents with diabetes: a review of indwelling catheters.* Pediatr Diabetes, 2004. **5**(2): p. 102-11.

172. Weissberg-Benchell, J., et al., *Adolescent diabetes management and mismanagement.* Diabetes Care, 1995. **18**(1): p. 77-82.

173. Schober, E., et al., *Prevalence of intentional under- and overdosing of insulin in children and adolescents with type 1 diabetes.* Pediatr Diabetes, 2011. **12**(7): p. 627-31.

174. Morris, A.D., et al., *Adherence to insulin treatment, glycaemic control, and ketoacidosis in insulin-dependent diabetes mellitus. The DARTS/MEMO Collaboration. Diabetes Audit and Research in Tayside Scotland. Medicines Monitoring Unit.* Lancet, 1997. **350**(9090): p. 1505-10.

175. American Diabetes, A., *Economic costs of diabetes in the U.S. In 2007.* Diabetes Care, 2008. **31**(3): p. 596-615.

176. Liebl, A., *Challenges in optimal metabolic control of diabetes.* Diabetes Metab Res Rev, 2002. **18 Suppl 3**: p. S36-41.

177. Asche, C.V., L. Shane-McWhorter, and S. Raparla, *Health economics and compliance of vials/syringes versus pen devices: a review of the evidence.* Diabetes Technol Ther, 2010. **12 Suppl 1**: p. S101-8.

178. Kreugel, G., et al., *Randomized trial on the influence of the length of two insulin pen needles on glycemic control and patient preference in obese patients with diabetes.* Diabetes Technol Ther, 2011. **13**(7): p. 737-41.

179. Burdick, P., et al., *Use of a subcutaneous injection port to improve glycemic control in children with type 1 diabetes.* Pediatr Diabetes, 2009. **10**(2): p. 116-9.

180. Donnelly, R.F., T.R. Raj Singh, and A.D. Woolfson, *Microneedle-based drug delivery systems: microfabrication, drug delivery, and safety.* Drug Deliv, 2010. **17**(4): p. 187-207.

181. Haq, M.I., et al., *Clinical administration of microneedles: skin puncture, pain and sensation.* Biomed Microdevices, 2009. **11**(1): p. 35-47.

182. Elleri, D., D.B. Dunger, and R. Hovorka, *Closed-loop insulin delivery for treatment of type 1 diabetes.* BMC Med, 2011. **9**: p. 120.

183. McVey, E., et al., *Pharmacokinetics and Postprandial Glycemic Excursions following Insulin Lispro Delivered by Intradermal Microneedle or Subcutaneous Infusion.* J Diabetes Sci Technol, 2012. **6**(4): p. 743-54.

184. Sharma, R., et al., *Quantitative imaging of lymph function.* Am J Physiol Heart Circ Physiol, 2007. **292**(6): p. H3109-18.

185. Nucci, G. and C. Cobelli, *Models of subcutaneous insulin kinetics. A critical review.* Comput Methods Programs Biomed, 2000. **62**(3): p. 249-57.

186. Vaughn, D.E. and D.B. Muchmore, *Use of recombinant human hyaluronidase to accelerate rapid insulin analogue absorption: experience with subcutaneous injection and continuous infusion.* Endocr Pract, 2011. **17**(6): p. 914-21.

187. Raz, I., et al., *Effect of a local heating device on insulin and glucose pharmacokinetic profiles in an open-label, randomized, two-period, one-way crossover study in patients with type 1 diabetes using continuous subcutaneous insulin infusion.* Clin Ther, 2009. **31**(5): p. 980-7.

188. Patel, S.R., et al., *Targeted administration into the suprachoroidal space using a microneedle for drug delivery to the posterior segment of the eye.* Invest Ophthalmol Vis Sci, 2012. **53**(8): p. 4433-41.

189. Scherliess, R., *Delivery of antigens used for vaccination: recent advances and challenges.* Ther Deliv, 2011. **2**(10): p. 1351-68.

190. Kresse, H. and H. Rovini, *Influenza vaccine market dynamics.* Nat Rev Drug Discov, 2009. **8**(11): p. 841-2.

191. Kochanek, K.D., et al., *Deaths: preliminary data for 2009.* National Vital Statistics Reports, 2011. **59**(4).

192. Centers for Disease Control and Prevention, *Estimates of deaths associated with seasonal influenza --- United States, 1976-2007.* MMWR Morb Mortal Wkly Rep, 2010. **59**(33): p. 1057-62.

193. Centers for Disease Control and Prevention, *Adult vaccination coverage--United States, 2010.* MMWR Morb Mortal Wkly Rep, 2012. **61**(4): p. 66-72.

194. Taddio, A., et al., *Survey of the prevalence of immunization non-compliance due to needle fears in children and adults.* Vaccine, 2012. **30**(32): p. 4807-12.

195. Harris, K.M., et al., *Seasonal Flu Vaccination: Why Don't More Americans Get It?*, in *Research Briefs*2011, RAND Corporation.

196. Johnson, D.R., K.L. Nichol, and K. Lipczynski, *Barriers to adult immunization.* Am J Med, 2008. **121**(7 Suppl 2): p. S28-35.

197. Uscher-Pines, L., et al., *Healthy young and middle age adults: what will it take to vaccinate them for influenza?* Vaccine, 2010. **28**(46): p. 7420-2.

198. Weniger, B.G. and M.J. Papina, *Alternative Vaccine Delivery Methods*, in *Vaccines*, S.A. Plotkin, W.A. Orenstein, and P.A. Offit, Editors. 2008, Elsevier: Philadelphia. p. 1357-1392.

199. Fondation Meriux, *Report of the Meeting on Typhoid Fever, a Neglected Disease: Towards a Vaccine Introduction Policy*, 2007: http://www.who.int/immunization/sage/typhoid_meeting_july.pdf.

200. Kommareddy, S., et al., *Dissolvable microneedle patches for the delivery of cell-culture-derived influenza vaccine antigens.* J Pharm Sci, 2012. **101**(3): p. 1021-7.

201. Sullivan, S.P., et al., *Dissolving polymer microneedle patches for influenza vaccination.* Nat Med, 2010. **16**(8): p. 915-20.

202. Zhang, Y., et al., *Development of lidocaine-coated microneedle product for rapid, safe, and prolonged local analgesic action.* Pharm Res, 2012. **29**(1): p. 170-7.

203. Choi, H.J., et al., *Stability of influenza vaccine coated onto microneedles.* Biomaterials, 2012. **33**(14): p. 3756-69.

204. Arnou, R., et al., *Willingness to vaccinate or get vaccinated with an intradermal seasonal influenza vaccine: a survey of general practitioners and the general public in France and Germany.* Adv Ther, 2011. **28**(7): p. 555-65.

205. Prymula, R., et al., *Acceptance and opinions of Intanza/IDflu intradermal influenza vaccine in the Czech Republic and Turkey.* Adv Ther, 2012. **29**(1): p. 41-52.

206. Arnou, R., et al., *Immunogenicity, large scale safety and lot consistency of an intradermal influenza vaccine in adults aged 18-60 years: Randomized, controlled, phase III trial*. Hum Vaccin, 2010. **6**(4): p. 346-54.

207. Arnou, R., et al., *Intradermal influenza vaccine for older adults: a randomized controlled multicenter phase III study.* Vaccine, 2009. **27**(52): p. 7304-12.

208. Coleman, B.L., et al., *A randomized control trial comparing immunogenicity, safety, and preference for self- versus nurse-administered intradermal influenza vaccine.* Vaccine, 2012.

209. Gill, H.S. and M.R. Prausnitz, *Coated microneedles for transdermal delivery.* J Control Release, 2007. **117**(2): p. 227-37.

210. Raymond, H.F., et al., *Comparing internet-based and venue-based methods to sample MSM in the San Francisco Bay Area.* Aids and Behavior, 2010. **14**(1): p. 218-24.

211. Frew, P.M., et al., *Factors mediating seasonal and influenza A (H1N1) vaccine acceptance among ethnically diverse populations in the urban south.* Vaccine, 2012. **30**(28): p. 4200-8.

212. Radosevic, K., et al., *Heterologous prime-boost vaccinations for poverty-related diseases: advantages and future prospects.* Expert Rev Vaccines, 2009. **8**(5): p. 577-92.

213. Lu, S., *Heterologous prime-boost vaccination.* Curr Opin Immunol, 2009. **21**(3): p. 346-51.

214. Kapitza, C., et al., *Basal-prandial insulin delivery in type 2 diabetes mellitus via the V-Go: a novel continuous subcutaneous infusion device.* J Diabetes Sci Technol, 2008. **2**(1): p. 40-6.

215. *Global WHO Alliance for the Elimination of Blinding Trachoma by 2020.* Wkly Epidemiol Rec, 2012. **87**(17): p. 161-8.

216. Levison, M.E., *Pharmacodynamics of Antimicrobial Drugs.* Infectious Disease Clinics of North America, 2004. **18**: p. 451-465.

217. Rothman, K.J., S. Greenland, and T.L. Lash, *Modern epidemiology*. 3rd ed. 2008, Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins. x, 758 p.

218. Searcy, C.P., et al., *Pharmacological prevention of combat-related PTSD: a literature review.* Mil Med, 2012. **177**(6): p. 649-54.

219. Grice, E.A. and J.A. Segre, *The skin microbiome.* Nat Rev Microbiol, 2011. **9**(4): p. 244-53.

220. Lee, K. and D. Kaplan, *Tissue engineering II : basics of tissue engineering and tissue applications*. Advances in biochemical engineering/biotechnology. 2007, Berlin ; New York: Springer. x, 335 p.

221. Behavioral Surveillance Team, *National HIV Behavioral Surveillance among Heterosexuals at Risk of HIV Infection: Venue-based Sampling Operations Manual*, 2006, Centers for Disease Control and Prevention.

222. Kotz, S. and J.R. Van Dorp, *Beyond beta : other continuous families of distributions with bounded support and applications*. 2004, Singapore ; Hackensack, NJ: World Scientific. xvi, 289 p.

VITA

James J. Norman

James was born in San Antonio, Texas. He received a B.S. in Chemical Engineering from the University of Texas at Austin prior to coming to Georgia Tech. Although devoted improving public health at work, he occasionally finds time to hike, bike, and play capoeira, go & chess.